EFFECTS OF FAR-ULTRAVIOLET RADIATION AND OXYGEN ON MACROMOLECULAR SYNTHESIS AND PROTEIN INDUCTION IN *BACTEROIDES FRAGILIS* BF-2

BY

JACOBA PETRONELLA SCHUMANN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF SCIENCE, UNIVERSITY OF CAPE TOWN

NOVEMBER 1983

"We need to entertain every prospect of novelty, every chance that could result in new combinations. But at the same time we need to entertain those with sceptical examination, and subject them to the most impartial scrutiny, for the probability is that nine hundred and ninety-nine of them will come to nothing, either because worthless in themselves or because we shall not know how to elicit their value; but we had better entertain them all, however sceptically, for the thousandth idea may be the one that will change the world."

"Dialogues of Alfred North Whitehead,
as recorded by Lucien Price,"
Little, Brown & Co., Boston,
pp. 284-285, 1954.

CERTIFICATION OF SUPERVISORS

In terms of paragraph 9 of "General regulations for the Degree of Ph.D." we, as supervisors of the candidate, J.P. Schumann, certify that we approve of the incorporation in this thesis of material that has already been published or submitted for publication.

Professor D.R. Woods
Head
Department of Microbiology

Signed by candidate

Dr T. Jones
Senior Lecturer Department
of Microbiolo

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Professor David R Woods, for his expert guidance and encouragement throughout this study, but most of all, for his infectious enthusiasm and explicit belief in the project. I also wish to thank my co-supervisor, Dr David T Jones, for his creative suggestions during this study.

To all the other members of the Microbiology Department I would like to express my gratitude for their interest, helpfulness and friendliness.

I am also deeply indebted to my mother and father for their never-failing emotional support in everything I did.

I would like to thank Jean Wilmot for the neat typing of this manuscript and wish to acknowledge financial assistance from the South African Council for Scientific & Industrial Research for the support of this work.

CONTENTS

Abstract			i
Chapter 1	:	General Introduction	1
Chapter 2	:	Effect of UV irradiation on macromolecular synthesis and viability in *Bacteroides fragilis*	27
Chapter 3	:	UV light induction of proteins in *Bacteroides fragilis* under anaerobic conditions	73
Chapter 4	:	Effect of oxygen and hydrogen peroxide on nucleic acid and protein synthesis in unirradiated and UV-irradiated *Bacteroides fragilis* cells	126
Chapter 5	:	Induction of proteins by oxygen and hydrogen peroxide in unirradiated and UV-irradiated *Bacteroides fragilis* cells	191
Chapter 6	:	Summary and Conclusion	231
Appendix I	:	Media and Solutions	243
References			256

ABSTRACT

This thesis deals with a study of the effects of far-UV radiation, oxygen and hydrogen peroxide on macromolecular synthesis and viability in the obligate anaerobe, *Bacteroides fragilis*, as well as the specific proteins induced in this organism by these different DNA damaging agents.

Irradiation of *Bacteroides fragilis* cells with far-UV light (254 nm) under anaerobic conditions resulted in the immediate, rapid and extensive degradation of DNA which continued for 40 to 60 min after irradiation. During the degradation phase, DNA synthesis was decreased but was never totally inhibited, and continued in a linear fashion for a dose-dependent period before exponential DNA synthesis resumed. DNA synthesis decreased with increasing UV fluences. DNA degradation after irradiation was inhibited by chloramphenicol and caffeine. DNA synthesis in irradiated cells was reduced by chloramphenicol but resumed after 100 min at the same rate as in irradiated cells without chloramphenicol. Irradiated cells continued to synthesize DNA for 40 min in the presence of caffeine but after this time DNA synthesis was completely inhibited and never recovered. RNA and protein synthesis were decreased by UV irradiation and the degree of inhibition

was proportional to the UV dose. Colony formation was not affected immediately by UV irradiation and continued for a dose-dependent period prior to inhibition. There was an inverse relationship between UV dose and inhibition of colony formation which occurred sooner in cells irradiated with lower doses of UV light.

Far-UV irradiation of *B.fragilis* cells under anaerobic conditions resulted in the induction of a new 95 000-molecular weight protein and the increased synthesis of two proteins with molecular weights of 90 000 and 70 000. The latter two proteins were synthesized in small amounts in unirradiated cells. The induction of a 37 000- to 40 000-molecular weight protein was not observed in irradiated *B.fragilis* cells. The synthesis of the UV-inducible proteins increased over a 35-min period following UV irradiation and then decreased with a halflife of 20 to 25 min once maximal levels were reached. Sublethal concentrations of caffeine reduced the survival of irradiated cells and specifically inhibited the induction of the 95 000-, 90 000- and 70 000-molecular weight proteins under anaerobic conditions. Sodium arsenite did not affect the induction of the three inducible proteins or the survival of irradiated *B.fragilis* cells. Both caffeine and sodium arsenite had very little or no effect on unirradiated *B.fragilis* cells.

Colony formation in exponential *B.fragilis* cells in minimal medium was inhibited when the cells were exposed to oxygen. After a period of time in oxygen there was a decrease in viability of the cells. DNA, RNA and protein synthesis in *B.fragilis* continued at a reduced rate for a limited period after exposure to oxygen, before it was inhibited. The rate and time of inhibition of macromolecular synthesis by oxygen was affected by the method of aeration. DNA synthesis was more sensitive to oxygen than RNA or protein synthesis. The plateau in incorporation of radiolabelled thymidine into DNA was not due to a shutoff of isotope transport, but represented a net inhibition of DNA synthesis. Once DNA synthesis was inhibited by oxygen, restoration of the cells to anaerobic conditions did not result in a resumption of synthesis. There was a correlation between the inhibition of DNA synthesis and the decrease in viability of cells exposed to oxygen. The effects of hydrogen peroxide on DNA synthesis and colony formation under anaerobic conditions were dependent on the concentration of hydrogen peroxide. At high concentrations of hydrogen peroxide DNA synthesis and colony formation were inhibited but no loss of viability occurred in hydrogen peroxide-treated *B.fragilis* cells. There was a synergistic effect between oxygen and hydrogen peroxide and concentrations of hydrogen peroxide which had no effect on DNA synthesis over 60 min under anaerobic conditions, inhibited DNA synthesis

immediately in the presence of oxygen.

DNA synthesis in UV-irradiated *B.fragilis* cells exposed to oxygen was completely inhibited and no degradation of DNA was observed. The rate and degree of inhibition of DNA synthesis in aerobically irradiated cultures was dose-dependent. RNA and protein synthesis were relatively unaffected after similar treatments. DNA synthesis in irradiated *B.fragilis* cells treated with hydrogen peroxide was characterized by a degradation phase followed by net DNA synthesis. The addition of caffeine or chloramphenicol did not affect the pattern of DNA synthesis in aerated unirradiated *B.fragilis* cells or in prelabelled cells irradiated under aerobic conditions.

Two proteins with molecular weights of 90 000 and 70 000 were induced by oxygen and hydrogen peroxide in unirradiated and UV-irradiated *B.fragilis* cells. These proteins were synthesized in small amounts in untreated cells. Caffeine inhibited the induction of the 90 000- and 70 000-molecular weight proteins by oxygen and hydrogen peroxide. A 95 000-molecular weight protein was induced by hydrogen peroxide and UV irradiation, but was inhibited by oxygen. Oxygen also inhibited the synthesis of a 40 000-molecular weight protein. Oxygen furthermore specifically induced the synthesis of a 37 000-molecular weight protein;

the synthesis of this protein was not induced by hydrogen peroxide or UV radiation under anaerobic conditions. A 106 000-molecular weight protein was induced by both oxygen and hydrogen peroxide.

The relationships between the DNA damage-induced proteins, macromolecular synthesis in damaged *B.fragilis* cells and the observed physiological responses and inducible repair phenomena after the different DNA damaging treatments in this anaerobe are discussed.

CHAPTER 1

GENERAL INTRODUCTION

The existence of anoxybiontic, aero-intolerant bacteria was first reported in 1861 by Louis Pasteur when he discovered these obligate anaerobic organisms during his studies on butyric fermentation processes. Ever since then, people have been intrigued by the very nature of obligate anaerobes and considerable effort has been expended in search of the prime cause for the adverse sensitivity of the anaerobes to molecular oxygen. Despite the restriction placed on anaerobes by their requirement for an anaerobic environment and availability of appropriate organic and inorganic electron donors and acceptors for energy generation, they are widely distributed in nature. Studies on the anaerobic bacteria inhabiting the gastro-intestinal tract of man and the rumen of herbivores have resulted in a growing awareness of the ecological importance of the anaerobic bacteria as causative agents of disease, especially in man. Furthermore, the potential of anaerobes in biotechnology is also focusing increasing attention on these bacteria. Although the technical difficulties involved in working with anaerobic bacteria have been responsible for their general neglect in the past and the great disparity that exists between

studies on these organisms and that of aerobes, modern technology has changed the situation and made the study of these organisms considerably easier.

Fundamental studies on the molecular genetics of anaerobes are important in furthering our understanding of the physiology, biochemistry and pathogenicity of these organisms, and in realizing the potential of recombinant DNA technology. The study of the genetics of anaerobes is still very much in its early initiation stage and this introduction gives an overview of genetic studies in *Bacteroides* strains with special reference to the work done in our laboratory on *Bacteroides fragilis*. More detailed introductions on the specific areas of the responses to UV irradiation addressed in this thesis, are given at the beginning of the relevant chapters.

The strain of *B.fragilis* utilized in all our studies, Bf-2, was isolated in 1977 from a clinical infection. The Gram-negative, non-sporeforming rod *Bacteroides fragilis* is the most common species of anaerobic bacteria isolated from human soft tissue infections (Moore & Holdeman, 1974) and the original description of *B.fragilis* by Veillon & Zuber in 1898 was based on strains isolated from abdominal abscesses following

appendicitis (Bergey's Manual, 8th Edition). Special attention has been focused on the occurrence and function of certain bile salt catabolic enzymes in *B. fragilis*, which are known to catalyze reactions which generate a variety of compounds which have been implicated in the etiology of cancer of the large intestines of man (Norman & Bergman, 1960; Hill *et al*, 1971).

The predominance of *B. fragilis* in clinical isolates suggests that it must possess unique virulence properties. This anaerobe only accounts for about 0,5% of the colonic microflora and is greatly outnumbered by the other *Bacteroides* species. Yet, in clinical isolates, *B. fragilis* accounts for 80% of all isolates. This virulence appears to be positively linked to the possession by about 80% of the isolated *B. fragilis* organisms of a high molecular weight polysaccharide layer external to the cell wall (Onderdonk *et al*, 1977), which is considered to be a cell surface antigen rather than a true capsule (Hofstad, 1979).

Apart from the fact that its medical importance demands further intensive research, Van Tassell & Wilkins (1978) suggested that *B. fragilis* is an appropriate choice for genetic studies of Gram-negative anaerobes because of certain of its physiological and biochemical characteristics. One of the favourable characteristics of

B.fragilis is the fair amount of oxygen tolerance exhibited by this anaerobe, allowing some manipulations to be done on the bench. Loesche (1969) found that *B. fragilis* can withstand exposure to room atmosphere for periods of at least 60 minutes without loss of viability, and Onderdonk *et al* (1976) reported that the effect of oxygen on *B.fragilis* cells growing in a chemostat is only bacteriostatic in nature.

A second advantageous characteristic of *B.fragilis* involves the relative ease with which phages specific for this organism can be isolated (Keller & Traub, 1974; Van Tassel & Wilkins, 1978), increasing the potential for obtaining transduction in this organism. Pseudolysogeny is very common in the *Bacteroides* group. Both Booth *et al* (1979) and Jones (1979) found a correlation between pseudolysogeny, phage resistence and encapsulation of *B.fragilis* cells, and Booth *et al* (1979) suggested that pseudolysogeny can be explained by the interference of the capsule with the attachment of phage to the *B.fragilis* cells. Keller & Traub (1974) reported that although the carrier cultures of *B.fragilis* adsorbed phage, these cells had an intracellular immunity to the injected DNA.

A third favourable characteristic of *B.fragilis* is its ability to grow well not only in complex media,

but also in simple defined medium supplemented with hemin (Quinto, 1966), suggesting that this bacterium possesses extensive biosynthetic capacities (Varel & Bryant, 1974). The requirement of *B.fragilis* for ammonia (produced by extensive breakdown of urea, amino acids and amino sugars in the colon) as nitrogen source, cannot be satisfied by amino acids in the medium: *B.fragilis* grows poorly, or not at all, when an amino acid mixture is used as the sole nitrogen source (Varel & Bryant, 1974). It is therefore often assumed that *B.fragilis* cannot utilize amino acids and Mossie *et al* (1979) reported that this organism is also unable to incorporate labelled amino acids. Smith & Salyers (1981), however, reported that there is some evidence that utilization of amino acids by *B.fragilis* can occur. During our studies we found that labelled methionine is readily incorporated into acid-precipitable material in *B.fragilis* cells.

B.fragilis presumably utilizes low molecular weight polysaccarides to satisfy its requirement for fermentable carbohydrates: Berg *et al* (1980) discovered that *B.fragilis* is a rich source of glycoside hydrolases, enzymes involved in the cleavage of such low molecular weight polysaccharides. Polysaccharides such as mucins and epithelial cell glycoproteins secreted by the host, as well as plant cell polysaccharides which cannot be

degraded by mammalian enzymes, appear to be the natural source of fermentable carbohydrates available to *Bacteroides* species in the colon (Salyers & Kotarski, 1980); however, Salyers *et al* (1977) suggested that colon cancer in man is brought about as a result of this polysaccharide fermentation by *Bacteroides* species.

The *Bacteroides* group of obligate anaerobes became a subject of interest when these organisms were identified as the causative agents in nearly all the post-operative infections and abscesses following abdominal surgery (Finegold, 1970; Felner & Dowell, 1971; Martin, 1971). The clinical treatment of *Bacteroides* infections is often very difficult because of their unusual resistance to a diversity of antibiotics, particularly aminoglycosides and beta-lactam drugs. These opportunistic pathogens are indigenous to the intestines of humans and other animals, and the emergence of an increasing number of antibiotic resistant bacteria isolated from clinical infections led to the speculation that *Bacteroides* strains act as reservoirs for antibiotic resistance factors in the gut. This speculation and the possibility that these antibiotic determinants may be situated on transferrable plasmids stimulated a study of these

genetic elements in *Bacteroides* strains.

At first, experimental results indicated that not only did *Bacteroides* strains harbour to transferrable plasmids, but that anaerobic conditions inhibited transfer of R plasmids (Fisher, 1957). Recently, however, reports contradicting both these statements have been published. Several *Bacteroides* strains were found to harbour plasmids (Stiffler *et al*, 1974; Guiney & Davis, 1975; Tinnell & Macrina, 1976) and especially *B.fragilis* was shown to contain several resident plasmids (Stiffler *et al*, 1974; Tinnell & Macrina, 1976). The correlation of molecular size and plasmid copy number found in *Bacteroides* strains was reminiscent of a variety of other plasmid systems seen in both Gram-negative and Gram-positive bacteria, but attempts to link the presence of plasmids to phenotypes such as drug resistance, bacteriocin production and virulence (Anderson & Sykes, 1973; Guiney & Davis, 1975) were not always successful.

Both Stallions & Curtiss (1972) and Moodie & Woods (1973) reported that anaerobic conditions did not necessarily inhibit plasmid transfer and showed that high frequency chromosome transfer and R plasmid transfer is possible in *Escherichia coli* under anaerobic conditions. Furthermore the successful

transfer of R plasmids from *E.coli* to *Bacteroides* strains (Burt & Woods, 1976), from *B.fragilis* to *E.coli* (Mancini & Behme, 1977; Guiney & Davis, 1978; Welch *et al*, 1979; Rashtchian & Booth, 1981) and between *Bacteroides* species (Privitera *et al*, 1979a; Tally *et al*, 1979) have also been reported.

Transfer of these R plasmids between *Bacteroides* strains requires cell-to-cell contact and occurs via a conjugation mechanism. Recently a novel feature regarding the conjugative transfer of antibiotic resistance in *B.fragilis* has been identified. Tally *et al* (1981) found that an increased transfer of R plasmid can be induced by pretreating the donor bacterium with antibiotics. They studied the transfer of the self-transmissible clindamycin resistance plasmid pBFTM 10 to the tetracycline-sensitive *B. fragilis* TMP 10 strain. Pretreating a tetracycline-resistant pBFTM 10 donor strain with clindamycin and tetracycline resulted in a 100- and a 1000-fold increase, respectively, of clindamycin and tetracycline resistance. Tetracycline resistance and clindamycin resistance were not linked and segregated independently in matings (Smith *et al*, 1982), suggesting that a complex relationship exists between the clindamycin transfer plasmid and the tetracycline resistance element in *B.fragilis* (Tally *et al*, 1981).

Privitera *et al* (1979b) found that the transfer of tetracycline resistance between *Bacteroides* species is also induced by growing the donor in tetracycline before mating, suggesting that tetracycline resistance is borne on a transferrable plasmid whose conjugative transfer requires activation of the antibiotic resistance genes and that both expression and conjugative ability involve a common regulatory system.

Present evidence, however, suggests a chromosomal location for the tetracycline resistance gene and recent reports on the transfer of tetracycline resistance revealed another novel feature of the conjugative transfer of antibiotic resistance in *B.fragilis*. Several investigators demonstrated the non-plasmid transfer of tetracycline resistance in these organisms (Mays *et al*, 1981; Tally *et al*, 1981; Mays *et al*, 1982; Rashtchian *et al*, 1982; Smith *et al*, 1982), which requires cell-to-cell contact and is inducible by and dependent on the presence of sub-inhibitory concentrations of tetracycline. No naked DNA or phage or phage-like particles are involved and the self-transmissible system is "transposon-like" in nature. Similar systems are suspected to operate in *Streptococcus faecalis* (Franke & Clewell, 1981) and *Clostridium difficile* (Smith *et al*, 1981) and can be described as conju-

gative transposons. These conjugative transposons are small, coding only for a few genes, and are of obvious medical importance, judging from the high incidence of tetracycline resistance amongst clinical isolates (Rashtchian *et al*, 1982). The discovery of this inducible conjugative antibiotic resistance transposon system in *B.fragilis* emphasizes the dangers of prolonged and injudicious subtherapeutic and therapeutic use of antibiotics and the impact it can have on the spread of antibiotic resistance in anaerobic bacteria.

Although transposon-like site-specific recombination has been shown to occur in *B.fragilis*, no homologous recombination system has as yet been demonstrated in this organism. Attempts to transform this organism by chromosomal and plasmid DNA or to isolate a prophage which integrates into the bacterium's chromosomé, have been unsuccessful, and although several auxotrophic *B.fragilis* mutants containing stable chromosomal markers (Van Tassell & Wilkins, 1978) are available, no chromosomal mapping technique has been reported to date. This suggests that the process of genetic recombination in anaerobes is not similar to that reported for *E.coli*, which tends to be accepted as typical for bacteria in general.

In *E.coli* the pleiotropic *recA*$^+$ protein is responsible for homologous recombination processes. This important protein is inducible to high levels by DNA damaging agents and much of the current information indicates that several common enzymatic steps and pathways are involved in the process of general genetic recombination and in DNA repair. A study of induced DNA repair processes in anaerobes may thus be informative for optimizing recombination processes in these organisms if they were found to be present.

Studies with *E.coli* have provided all the initial insight into the molecular basis of DNA repair, especially in response to UV-induced damage: a brief overview of the *E.coli* repair systems is thus given as an introduction to the topic of DNA repair processes.

The major lesions induced by far-UV light in *E.coli* cells are intrastrand pyrimidine dimers (Beukers & Berends, 1961; Ben-Hur & Ben-Ishai, 1968). These dimers can act as blocks to normal DNA replication by inhibiting normal base pairing opposite dimer sites. Two distinct recovery schemes are operating in *E.coli* (Hanawalt *et al*, 1981), but the same biological end point (i.e. survival) results from either scheme. The one recovery scheme effects

the enzymatic removal of the damage from the DNA (i.e. repair), whereas the other repair scheme facilitates tolerance of the persisting damage in the genome by additional rounds of chromosome replication (Hanawalt *et al*, 1979).

The repair of pyrimidine dimer lesions occurs either by photoreactivation or by excision repair pathways.

(a) Photoreactivation

Photoreactivation brings about the direct reversal of pyrimidine dimer damage. A single cellular enzyme specifically binds to cyclobutyl pyrimidine dimers to generate a new enzyme-DNA chromophore (Harm, 1975; Rupert, 1975). This complex can form in the absence of light, but activation of the complex to catalyze the cleavage of the joined bases to monomerize pyrimidines requires the absorption of visible light of wavelength 320 to 410 nm (Rupert, 1975). This mechanism does not break any phosphodiester bonds and is error-free.

(b) Excision repair

Although the principle of excision repair is relatively simple, the interrelationship of the different enzymes

involved in excision repair is more complicated and no single mutation abolishes the entire excision repair process (Hanawalt *et al*, 1979). Base excision repair in *E.coli* is a sequential process initiated by a very specific N-glycosylase enzyme which recognizes a particular altered or incorrect (non-conventional) base, and cleaves this base from its sugar to generate an apurinic or apyrimidinic (AP) site (Friedberg *et al*, 1978 and 1981). The AP site may then be recognized by a specific AP endonuclease which cleaves the phosphodiester bond and excises the base-free sugar residue (Mosbaugh & Linn, 1980). This lesion is repaired through direct replacement of the missing base by an "insertase" mechanism (Hanawalt *et al*, 1981).

The nucleotide excision repair of pyrimidine dimer lesions requires at least four different enzyme actions. The repair sequence is initiated by the enzymatic recognition of the damage. A specific endonuclease then cuts the damaged DNA adjacent to the dimer (incision), followed by the removal of the region containing the dimer by an exonuclease (excision). Excision is followed by resynthesis, during which a polymerase enzyme reconstitutes the deleted stretch, using the opposite intact complementary strand as a template (Hanawalt *et al*, 1979). This template

requirement explains why excision repair is only possible in duplex regions of the DNA. Excision and resynthesis are closely coupled processes and Hanawalt *et al* (1981) proposed that concurrent polymerization may drive the excision step. Completion of excision repair requires the sealing of phosphodiester bonds by a polynucleotide to restore strand continuity (Hanawalt *et al* 1979).

Seeberg (1981) speculated that the ATP-dependent *uvrA*$^+$ protein is responsible for the initial recognition of the damage by identifying major regions of distortion of the DNA helix and binding to them. The complex formed by the *uvrB*$^+$ and *uvrC*$^+$ proteins binds to the *uvrA*$^+$ protein-DNA complex and this complex then facilitates excision of the dimers. The *uvrC*$^+$ protein apparently also interacts with the polynucleotide ligase system to prevent abortive sealing of incision breaks (Seeberg & Rupp, 1975).

The repair synthesis following the incision/excision step can occur via three major pathways which are linked to the somewhat different substrate requirements of the three polymerases involved in the resynthesis step of excision repair. Polymerase I can bind to nicked DNA, but polymerases II and III require the expansion of the nick to a gap by exonucleases before they will

bind to the damaged DNA and initiate polymerization (Hanawalt *et al*, 1981).

The one major excision repair pathway produces only short patches of repair synthesis (about 20 nucleotides long) and accordingly has been termed the short patch repair pathway (Cooper & Hanawalt, 1972a). This pathway requires a functional polymerase I enzyme (*polA* gene product), is independent of nutrients and is constitutively expressed (Cooper & Hunt, 1978). Short patch repair is error-free and is presumably responsible for most of the excision repair in *E.coli* (Youngs *et al*, 1974; Hanawalt *et al*, 1979), including LHR and HCR.

The second type of excision repair results in somewhat larger average patch sizes, is constitutive and is performed by either polymerase II or polymerase III (Hanawalt *et al*, 1981). The larger patch sizes may be connected with the intrinsic DNA binding requirements of these two polymerases, and this type of repair was found to be dependent on functional *recB* and *recC* gene products (Smith, 1978a). Youngs *et al* (1974) reported that polymerase III is capable of repairing about 25% of the lesions in the absence of a functional polymerase I enzyme. Evidence suggests that the *E.coli* *uvrD* gene product is involved in the regulation of either DNA polymerase II or III (Kushner *et al*, 1978).

The third kind of excision repair is called long-patch repair and produces patches of resynthesis of several hundred nucleotides long (Hanawalt *et al*, 1981). This repair is inducible and growth-medium-dependent; post-irradiation treatment of the cells with chloramphenicol irreversibly inhibits long-patch excision repair (Youngs *et al*, 1974; Cooper, 1981). It requires functional *recA* and *lexA* gene products in addition to uvr^+ proteins (Cooper & Hanawalt, 1972b; Youngs & Smith, 1973; Hanawalt *et al*, 1981). The protein synthesis required for long-patch repair presumably involves the enhanced production of the $uvrA^+$ and $uvrB^+$ protein, which were recently shown to be inducible by DNA damage (Fogliano & Schendel, 1981; Kenyon & Walker, 1981). Mutations in the *recB* and *recC* genes do not affect this type of excision repair and no one of the three polymerases appears to be essential for inducible long-patch repair. Polymerase II and/or polymerase III usually facilitates the repair of longer patches, but inducible long-patch repair can still occur in a *polB polC* mutant, indicating that polymerase I may be capable of long-patch repair under inducing conditions (Hanawalt *et al*, 1981).

The inducible long-patch repair system only operates on a defined minor class of damaged lesions, but the ability to perform inducible long-patch repair is apparently important for cell survival and also seems

to be an important component of the Weigle reactivation system (Cooper, 1981). The remaining component of Weigle reactivation in a *umuC* mutant is abolished by a *uvrA* mutation (Walker & Dobson, 1979; Sancar *et al*, 1982). It is proposed that this type of repair is required where DNA damage includes many dimers in close proximity to each other and the increased patch size may thus reflect the extensive excision and resynthesis required to repair such bulky lesions.

Although the polymerase I-dependent short patch repair is error-free, a considerable amount of UV-induced mutations have been attributed to longer-patch excision repair (Hanawalt *et al*, 1979). The results of Bridges & Mottershead (1978) indicated that mutagenic excision repair is constitutively expressed and involves polymerase III-dependent excision repair. A certain amount of error-free inducible long-patch excision repair has also been observed (Cooper, 1981), which includes the error-free Weigle reactivation reported by Rothman *et al* (1979).

(c) Postreplication repair

In *E.coli* a repair system that allows the organism to tolerate persisting damage, is induced by the damage itself (Hanawalt *et al*, 1979). This repair system

allows the bypassing of dimer sites during replication, with a high probability of error (Castellazzi *et al*, 1980), and is also responsible for the filling of gaps left in daughter strands opposite dimers when replication proceeds past a dimer lesion in the parental strand. Both bypass and gapfilling processes promote recovery although they do not remove the DNA damage. A number of gene products have been shown to be involved in the two components of "postreplication repair" and include *recA*$^+$, *lexA*$^+$, *recB*$^+$, *recC*$^+$, *uvrD*$^+$, *polA*$^+$ and *polC*$^+$ proteins (Smith, 1978a).

Gapfilling occurs via parent-daughter strand exchanges with intact homologous DNA from the isopolar parental strand (Hanawalt *et al*, 1979). The *recA*$^+$ protein participates directly in this process and the induced synthesis of *recA*$^+$ protein consequently leads to enhanced postreplication repair (Witkin, 1976). The daughter-strand gap repair is often referred to as recombinational repair because of its dependence on the participation of *recA*$^+$ protein, which is known to be responsible for general genetic recombination processes in *E.coli* cells; a *recA* mutation completely inhibits postreplication gap-filling (Smith & Meun, 1970). During this postreplication recombination process the pyrimidine dimers are randomly transferred to the daughter strands as gaps are repaired (Ganesan, 1974), and the dimers eventually become equally distributed amongst parental and progeny strands. In

an excision-deficient mutant several rounds of replication are required to gradually dilute out the dimers sufficiently to yield a dimer-free DNA copy.

Error-prone replication past dimers requires the action of either the polymerase I or the polymerase III enzymes: *polA* and *polC* single mutants can efficiently carry out postreplication repair, but this type of repair is absent in a *polA polC* double mutant (Sedgwick & Bridges, 1974; Tait *et al*, 1974). This transdimer synthesis is proposed to require the inactivation of the 3' → 5' editing exonuclease activity associated with bacterial polymerases by an inducible protein as part of the SOS response (Clark & Volkert, 1978). Bridges (1978) studied the involvement of the polymerase III enzyme in this mutagenic transdimer DNA synthesis. The polymerase III-dependent transdimer synthesis appears to be involved in the repair of only a few daughter-strand gaps and this fraction may be too small to detect biochemically under normal postreplication conditions; it is, however, large enough to account for the mutagenesis associated with postreplication repair (Hanawalt *et al*, 1979). Daughter-strand gap-filling by sister strand exchange is not involved in the mutagenesis, since the induced mutagenesis is observed in a *uvr recB recF* mutant which is deficient in all the recombination pathways (Kato *et al*, 1977).

Evidence is accumulating, however, that the DNA repair knowledge gained with the model bacterium, *E.coli*, is not necessarily of general validity and our results with the anaerobe *B.fragilis* also reflects this. We considered a study of DNA repair mechanisms in *B. fragilis* as an appropriate way of approaching genetic studies (for the establishment of a genetic system) in this organism. The effect of far-UV irradiation on bacterial DNA has been the most extensively studied and this damaging agent was thus the obvious choice for DNA repair studies in *B.fragilis*. Our research was initiated by a study of the physiological responses of *B.fragilis* to DNA damage and the work reported on here was done in our laboratory by Jones *et al* (1980), Jones & Woods (1981), Slade *et al* (1981), and Slade *et al* (1983a and 1983b).

The effect of far-UV irradiation (254 nm) on *B.fragilis* was found to be interesting as the bacterium is more sensitive to far-UV radiation under aerobic conditions than under anaerobic conditions. In all other bacteria studied so far the inactivation by far-UV light has been repeatedly shown to be independent of the presence of oxygen (Zetterberg, 1964; Webb & Lorenz, 1970; Webb, 1977). The presence of oxygen resulted in a decrease in the shoulder regions of the UV survival curves, but caused little or no change in the final slopes of the

curves; this suggested that the increase in sensitivity under aerobic conditions was not due to an increase in photochemical DNA damage, but was due to a difference in the efficiency of repair processes under anaerobic and aerobic conditions. Support for this came from the finding that the number of pyrimidine produced by UV irradiation under anaerobic and aerobic conditions were very similar.

The increased UV sensitivity of *E.coli* B and *E.coli* K12 *lon* mutants is due to the inducible process of filament formation in the majority of the cells after exposure of the cells to even small UV doses (Witken, 1967 and 1976). In *B.fragilis* filament production was dose-dependent and the *B.fragilis* filaments differed from *E.coli* filaments in that they always contained a certain number of septa. This difference in filament formation between *E.coli* strains and *B.fragilis* and the shape of the survival curve obtained with *B.fragilis* cells, suggests that the differences in the survival of irradiated *B.fragilis* cells under different conditions are not directly related to filament formation.

Since cellular metabolism and photochemical oxidation in the environment generate a variety of derivatives of oxygen, investigations were carried out to determine whether the increase in UV sensitivity of *B.fragilis*

cells under aerobic conditions was due to oxygen itself or to a derivative of oxygen. It was found that the sensitization is a specific effect of molecular oxygen and that treatment of the cells with hydrogen peroxide prior to irradiation actually increased the survival of irradiated cells. It was also concluded that *B.fragilis* cells are more sensitive to far-UV irradiation under aerobic conditions rather than being more sensitive to oxygen after UV irradiation.

The survival level of irradiated *B.fragilis* cells under aerobic conditions was midway between those obtained for the repair-competent and repair-deficient (*uvrA*$^-$) strains of *E.coli*. There was a marked decrease in survival when *B.fragilis* cells were plated onto caffeine plates after irradiation under both anaerobic and aerobic conditions. Caffeine inhibits excision repair processes in *E.coli* (Rupert & Harm, 1966; Setlow, 1967; Swenson, 1976; Fong & Bockrath, 1979; Rothman, 1980) and although it is not known whether the mechanisms of action of caffeine in *B.fragilis* are similar to those reported for *E.coli*, the results of the caffeine experiments suggested that excision repair processes might function under both anaerobic and aerobic conditions. The decreased survival of UV-irradiated cells under aerobic conditions is therefore not only due to decreased excision of DNA damage.

No photoreactivation was observed in UV-irradiated *B.fragilis* cells, irrespective of whether the cells were irradiated under anaerobic or aerobic conditions. Photoreactivation was also found to be absent in a number of other bacteria (Rupert, 1975) and in *E.coli* anaerobic conditions repressed this type of repair of UV damage (Tyrrell, 1973).

Liquid holding recovery (LHR) of buffer-held UV-irradiated *B.fragilis* cells was observed only under aerobic conditions: anaerobic conditions totally inhibited this recovery. This was also a molecular oxygen-specific phenomenon, since hydrogen peroxide treatment of irradiated cells under anaerobic conditions did not enhance LHR. LHR was nutrient-independent, but temperature-dependent and the rate and amount of recovery was optimal at 37°C. In *E.coli* LHR is dependent on functional *uvr* (Ganesan & Smith, 1969; Aragão *et al*, 1980) and *polA* (Tang & Patrick, 1977b) gene products, and is only observed in *E.coli* K12 *recA* mutants and *E.coli* B cells (Smith, 1978a). Caffeine and acriflavine completely inhibited LHR in both *E.coli* (Swenson, 1976) and *B.fragilis* cells, suggesting that LHR in *B.fragilis* may be due to a similar *polA*-dependent excision repair process as in *E.coli*.

Since the repair of irradiated phages which do not code for their own repair functions depends primarily

on the repair capabilities of the host bacterium (Bernstein, 1981) the repair of far-UV-irradiated phage b-1 after infection of *B.fragilis* cells was studied in an attempt to elucidate repair processes in this anaerobe. In *E.coli* host cell reactivation of irradiated phage lambda is entirely dependent on the constitutive excision repair processes of the host (Bernstein, 1981). Weigle-like reactivation of UV-irradiated phage b-1 was demonstrated in *B.fragilis* under anaerobic conditions. In addition to this, two other interesting phage reactivation systems have been shown to operate in *B.fragilis* cells. A study of the survival or irradiated phage b-1 in *B.fragilis* cells revealed an increased recovery of the irradiated phage if it was plated on exponential phase cells which had been exposed to air for at least 15 to 20 minutes. This phage reactivation was not inducible in stationery phase cells. Adsorption of the phage b-1 did not play a role in the oxygen-induced phage reactivation, and neither did the irradiation of the phage under anaerobic and aerobic conditions affect the survival levels obtained. The addition of chloramphenicol or caffeine before exposing the cells to air totally inhibited the induction of the oxygen-inducible phage reactivation system.

Hydrogen peroxide also induced a phage reactivation

system in *B.fragilis*, but it differed from the oxygen-induced system. The induction of phage reactivation by hydrogen peroxide was immediate and removal of hydrogen peroxide by washing only caused a slight decrease in the induced survival level of the irradiated phage over 90 minutes. The biological half-life of the oxygen-induced phage reactivation system was only 15 minutes. The addition of chloramphenicol and caffeine before exposing the *B.fragilis* cells to hydrogen peroxide reduced the level of the induced phage reactivation, but never totally inhibited it. Furthermore, the hydrogen peroxide-induced phage reactivation system was not affected by the growth phase and the level was consistently higher than that obtained with oxygen.

Although the responses of *B.fragilis* to DNA damage are relatively well-defined on a physiological level, no studies have as yet been reported on the effect of oxygen, oxygen derivatives and far-UV irradiation on DNA, RNA and protein synthesis in *B.fragilis*, or on the induction of proteins by these DNA damaging agents. Such molecular studies may allow a more meaningful evaluation of certain of the observations for which we have no ready explanation as yet. Investigations on DNA repair systems in *B.fragilis* are at present

hampered by our inability to isolate UV-sensitive or resistant mutants in spite of an extensive screening program. Our studies thus relate in many aspects to similar studies done in *E.coli* in the 50's and 60's when the study of UV repair processes was initiated in this organism. The basic studies on *B.fragilis* reported in this thesis nevertheless provide a starting point for future work on UV repair and recombination in this important anaerobe, as well as characterizing the toxic effect of oxygen in this organism on a molecular level.

CHAPTER 2

EFFECT OF UV IRRADIATION ON MACROMOLECULAR SYNTHESIS AND VIABILITY IN *BACTEROIDES FRAGILIS*

2.1 INTRODUCTION

The continued proliferation of living organisms and concomitant maintenance of their inherent genetic identity from one generation to the next, depend on the stable and precise replication of their DNA. DNA, however, is the primary target for far-UV irradiation (254 nm), with induced intrastrand 5,6-cyclobutyl-dipyrimidines, commonly called pyrimidine dimers (Smith, 1978b), recognized to be the most important biological lesions following this type of radiation (Setlow & Carrier, 1966; Ben-Hur & Ben-Ishai, 1968). These lesions structurally distort the DNA double helix and thus tend to interfere with the normal synthesis of DNA and RNA, and consequently, with normal protein synthesis. Living cells, however, possess the important capability of repairing their damaged DNA: limited degradation of the damage-containing DNA is followed by local repair synthesis in which the double helix is reconstructed at each repair site; alternatively, replication can proceed past dimer sites with an expected high frequency of error.

The effect of UV irradiation on the kinetics of

incorporation of radioactive precursors into DNA, RNA and protein during the period after irradiation when extensive DNA repair is thought to occur, can reveal much about the nature of the functional repair systems in organisms. This knowledge is supplemented by a study of the effect of specific repair protein inhibitors on DNA replication in irradiated cells. Studies with mutants of the aerobe *E.coli* have provided most of the initial insight into macromolecular synthesis and the molecular basis of repair processes in irradiated organisms. Although several UV-induced repair phenomena have been reported for anaerobes (Jones & Woods, 1981; Slade *et al*, 1983b), there appears to be no information available on the effect of far-UV radiation on macromolecular synthesis and viability in anaerobes.

2.1.1 Degradation of DNA

Partial or limited degradation of irradiated DNA is an essential part of the DNA repair process in *E.coli* cells: excessive DNA degradation or loss of DNA breakdown is usually associated with the loss of normal repair capabilities (Howard-Flanders & Boyce, 1966). This degradation results from processes such as the excision of damage-containing DNA regions and gap expansion for the binding of polymerases II and III. UV irradiation of

repair-competent *E.coli* B/r and K12 cells results in about a 10% loss of acid-insoluble material from the DNA of these organisms (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964).

E.coli cells utilize at least three enzymes for excision of damaged regions from irradiated DNA. The ATP-dependent *recBC*-coded 5'→3' exonuclease V (Oishi, 1969; Goldmark & Linn, 1970; Lieberman & Oishi, 1974) associated with polymerase I (Lehman & Chien, 1973) is responsible for most of the degradation induced by UV and X-irradiation in *E.coli* cells (Emmerson, 1968; Kato & Kondo, 1969; Youngs & Bernstein, 1973). However, over 10% of the irradiated DNA is still degraded even in strains that lack the $recBC^{+}$ enzyme (Youngs & Bernstein, 1973): degradation in *polA recB* mutants (Strike & Emmerson, 1974) may be attributed to either the 5'→3' exonuclease activity associated with polymerase III (Livingston & Richardson, 1975) or the single-strand specific exonuclease VII (Chase & Richardson, 1974; Hanawalt *et al*, 1979). Two of the excision enzymes in *E.coli* are associated with polymerase activity and suggests that the excision and resynthesis steps occur simultaneously, possibly by a type of "nick translation" mechanism (Hall & Mount, 1981).

Oishi & Smith (1978) suggested that the primary role

of the *recBC* DNase is the detection of unusual DNA structures, such as pyrimidine dimers, single-strand breaks in the chromosome or immobilized replication forks, and the subsequent degradation of the damaged DNA to generate "signals" for the induction of high levels of SOS components (Gudas & Pardee, 1975; Little & Hanawalt, 1977). In support of this suggestion it was found that the requirements for the induction of SOS events in *E.coli* include the degradation of irradiated DNA (Oishi *et al*, 1981).

Howard-Flanders & Boyce (1966) suggested that breakdown is initiated at single-strand cuts in the DNA (presumably made by excision repair enzymes). This fits with the finding that the type of DNA damage affects the timing of degradation of damaged DNA (Oishi *et al*, 1981): agents which create strand breaks, such as bleomycin and streptonigrin, cause immediate DNA degradation, while UV irradiation, which only modifies DNA bases (pyrimidime dimer formation), causes relatively slower degradation of DNA after a short lag period of about 20 minutes (Oishi *et al*, 1981).

The "reckless" and rapid degradation of irradiated DNA in *E.coli recA* mutants (Clark *et al*, 1966; Howard-Flanders & Boyce, 1966; Howard-Flanders & Theriot, 1966; Clark, 1973) reflects the action of an uncontrolled

nuclease. Experimental evidence suggests that the binding of the induced *recA*$^+$ protein to single-strand regions in the DNA protects irradiated DNA from excessive degradation by the *recBC*$^+$ DNase (Marsden *et al*, 1974; Gudas & Pardee, 1975; Williams *et al*, 1981). Other mutants that also degrade their DNA excessively after UV irradiation include the *lexA* mutant (Howard-Flanders & Boyce, 1966), *uvrD* mutant (Ogawa *et al*, 1968) *polA1* mutant (Boyle & Setlow, 1970) and *resA1* mutant (also deficient in polymerase 1)(Kato & Kondo, 1970). There is no relationship between DNA degradation and survival of UV-irradiated cells, and the multiple mutant *recA recB recC* has about the same radiosensitivity as the *recA* single mutant, although the degradation in the former is greatly reduced (Willetts & Clark, 1969).

2.1.2 DNA synthesis in UV-irradiated cells

One of the first effects of the formation of UV photoproducts in far-UV-irradiated *E.coli* cells, is the immediate and complete inhibition of DNA synthesis (Kelner, 1953; Hanawalt & Setlow, 1960; Setlow *et al*, 1963) at the site of the replication point (Billen, 1969; Bridges, 1972). Swenson & Setlow (1964) reported that this inhibition is permanent in radiation-sensitive *E.coli* strains which are unable to excise their pyrimidine dimers, while in strains with the

capacity for dimer excision and repair of the DNA, the duration of DNA inhibition is dose-dependent (Doudney, 1965; Rupp & Howard-Flanders, 1968; Smith, 1969), though it never exceeds 45 minutes, which is approximately equal to one division time in irradiated *E.coli* cells (Doudney, 1965).

It was once thought that pyrimidine dimers act as terminating lesions for semiconservative DNA replication, causing inhibition of DNA synthesis, and that the resumption of synthesis only occurs after dimers had been removed by excision repair processes (Setlow & Carrier, 1964; Swenson & Setlow, 1966). This belief was strengthened by the fact that photoreactivation could relieve the inhibition (Kelner, 1953; Setlow *et al*, 1963; Doudney, 1966) and the duration of inhibition was shorter in an excision-proficient than in an excision-deficient *E.coli* strain (Swenson & Setlow, 1966). Work done with excision-deficient mutants (Rupp & Howard-Flanders, 1968), however, showed that dimers are not absolute blocks for DNA replication (Smith, 1969). DNA synthesis can reinitiate downstream from the UV lesion and continues up to the next dimer where the process is repeated and carried out right through the whole irradiated genome. This leaves single-strand gaps (Moore *et al*, 1981) of approximately 1000 base pairs long in the newly

synthesized DNA opposite the pyrimidine dimers in the parental strand (Iyers & Rupp, 1971)(extending from the pyrimidine dimer site to the site of reinitiation of replication).

The gaps produced by the replicative bypassing of dimer sites are filled by a *recA*$^{+}$-dependent recombination mechanism in which homologous DNA from the sister chromosome is inserted into the gap (Bridges, 1972; Hall & Mount, 1981), transferring some of the dimers to daughter strands in the process (Rupp *et al*, 1971; Ganesan, 1974). *RecA*$^{-}$ cells synthesize short pieces of DNA, but fail to elongate them during subsequent incubation (Smith & Meun, 1970).

The inhibition of DNA synthesis in *E.coli* cells only occurs at higher UV fluences: this cessation of replication allows the excision repair processes to remove the more bulky type of DNA lesions resulting from high UV influence (Radman *et al*, 1970) without the concomitant interference of DNA replication and postreplication recombinational repair. At low fluences or other conditions where DNA replication is not inhibited, continued DNA replication and post-replication repair interfere in the excision of dimers in that bypassing of dimer sites leaves gaps in the newly synthesized DNA and the template dependency of

the excision repair system thus prevents the excision of dimers from the separated parental strands. One genetic exchange takes place for every one or two dimers present in irradiated DNA (Rupp *et al*, 1971) and this exchange may continue for as long as dimers persist in cells. In *E.coli uvr*$^-$ cells the pyrimidine dimers are never removed from acid-precipitable DNA (Howard-Flanders *et al*, 1966; Boyle & Setlow, 1970; Shlaes *et al*, 1974) and a dimer-free DNA copy of this mutant is only produced by the simple dilution of dimers amongst successive generations of DNA molecules synthesized after irradiation (Ganesan, 1974).

An interesting finding is that DNA synthesis is dependent on a functional *recA* gene product (Trgovčević *et al*, 1980) and Trgovčević *et al* (1980) suggested that the inhibition of DNA synthesis after DNA damage may be the first step in the sequential expression of the SOS event in *E.coli*. No immediate inhibition of DNA synthesis is observed in a *recA recB E.coli* mutant strain and the mutant carries on synthesizing DNA after irradiation at the same rate as in the unirradiated control for a dose-dependent time before synthesis is permanently inhibited (Trgovčević *et al*, 1980).

The filling of gaps in irradiated *E.coli* DNA after

excision of dimers requires normal semiconservative DNA synthesis and DNA replication resumes after a fluence-dependent lag at a rate which, below a certain "critical" dose, is equal to or slightly higher than that of the unirradiated control (Setlow *et al*, 1963; Doudney, 1965; Trgovčević *et al*, 1980). At higher fluences the rate of DNA synthesis is not restored to normal values and becomes progressively lower with increasing UV doses (Doudney, 1965; Trgovčević *et al*, 1980). Smith (1969) found that DNA synthesis in *E.coli* B/r irradiated up to about 16% survival resumes at essentially a normal unirradiated rate for about 60 minutes and then changes to a new rate that is proportional to the dose of UV radiation.

The reinitiation of semiconservative sequential DNA replication after UV irradiation occurs preferentially at the chromosome origin (Billin, 1969) and this "reinitiation recovery" requires *de novo* protein synthesis for a certain critical period after UV irradiation (Doudney, 1959; Drakulić & Errera, 1959; Bridges, 1972; Lark & Lark, 1978). Chloramphenicol and acriflavine added immediately after and rifampicin added within 13 minutes after UV irradiation prevent the resumption of DNA synthesis and decrease the fraction of surviving cells (Harold & Ziporin, 1958; Drakulić & Errera, 1959; Doudney *et al*, 1964; Doudney,

1965; Doudney *et al*, 1966; Doudney, 1973). Swenson & Setlow (1966), however, claimed that DNA synthesis in irradiated *E.coli* B/r cells resumed at the same time with or without chloramphenicol.

The DNA replication which resumes in *E.coli* cells after protein synthesis has been allowed for 40 minutes following UV irradiation (Kogoma *et al*, 1979) has an unique feature in that it can continue for several hours in the absence of further protein synthesis (Kogoma & Lark, 1970 and 1975; Lark & Lark, 1978). Normal DNA synthesis in unirradiated cells requires *de novo* protein synthesis for reinitiation of every new replication cycle (Maaløe & Hanawalt, 1961; Lark & Renger, 1969; Lark, 1972). Thymine and amino acid starvation prior to UV irradiation can elicit the same stable DNA replication response if protein synthesis is allowed between starvation and irradiation Sedliaková & Slezariková, 1977; Brozmanová & Sedliaková, 1980). The initiation of this abnormal stable DNA replication depends on a functional protein (Kogoma & Connaughton, 1978) and it is suggested that this form of DNA replication is part of the $recA^{+}lexA^{+}$-regulated SOS response (Brozmanová & Sedliaková, 1980; Hall & Mount, 1981). It is proposed that the functional $recA^{+}$ protein cleaves and inactivates a special termination protein (*dnaT* gene

product)(Lark & Lark, 1978). New replication complexes without termination factors are then assembled which promote initiation at chromosome origins and do not terminate replication at the end of the cycle. Chloramphenicol added after the resumption of the stable DNA replication has taken place, has no effect on DNA synthesis in irradiated cells (Lark & Lark, 1978), although chloramphenicol added to unirradiated cells causes DNA synthesis to cease within 60 minutes (Doudney, 1965; Lark & Lark, 1978).

2.1.3 RNA and protein synthesis in UV irradiated bacteria

RNA and protein synthesis in *E.coli* is much less sensitive to UV irradiation than DNA synthesis (Kelner, 1953) and may continue with linear kinetics during the period of DNA synthesis inhibition following UV irradiation (Hanawalt & Setlow, 1960; Swenson & Setlow, 1966). Similar differences in UV sensitivity have been observed with irradiated phages where large doses of UV irradiation inhibit the synthesis of T_{even} phage DNA, but the rate of the appearance of phage specific enzymes is not affected (Flaks *et al*, 1959; Dirksen *et al*, 1960; Delihas, 1961). Although pyrimidine dimers act as transcription-terminating lesions for RNA synthesis, the dimers in UV-irradiated DNA are sufficiently widely spaced to allow RNA

synthesis to occur between the lesions. This intra-dimer RNA synthesis accounts for the increase in production of shorter-than-normal RNA, and consequently, abnormally short polypeptides, observed in UV-irradiated *E.coli* cells (Brunschede & Bremer, 1969; Michalke & Bremer, 1969).

The synthesis of ribosomal RNA is more sensitive to UV irradiation than the synthesis of transfer RNA (Wainfan *et al*, 1963; Sibatani & Mizuno, 1963) and the differences in sensitivity may be directly linked to the different target sizes of the DNA templates (Kroes *et al*, 1963). The synthesis of the large 23S RNA in irradiated *E.coli* cells is therefore affected most readily with a corresponding inhibition of 50S ribosome synthesis which lasts for the whole period during which DNA synthesis is blocked (Sibatani & Mizuno, 1963). As soon as DNA synthesis resumes, RNA and protein synthesis increase proportionally (Swenson & Setlow, 1966).

There is a slight shoulder in the fluence-effect curve of RNA synthesis, but protein synthesis is decreased in a simple exponential way with increasing UV fluences (Hanawalt & Setlow, 1960). Several reports state that enzyme synthesis is inhibited by catabolite repression at the transcription level after UV irradiation of *E.coli* cells (Swenson, 1976). Experiments with the *lac*

operon indicated that pyrimidine dimers in DNA at sites other than the structural gene may cause repression of the *lac* operon and that this repression is only lifted once DNA repair is complete (Swenson, 1972).

2.1.4 Methodology in studying macromolecular synthesis

There appear to be a few pitfalls which should be avoided when DNA synthesis kinetics in UV-irradiated bacteria are measured. Smith & O'Leary (1968) emphasized the methodology of plotting the data in radioactive thymine- incorporation studies. They stated that linear plots of counts minute^{-1} *versus* time lead to fallacious conclusions, since the apparent kinetics of DNA synthesis under these conditions depend upon the number of cells per millilitre, on the specific activity of the radioactive precursor and on the DNA content and generation time of the cells. The true kinetics of the DNA synthesis only depend on the DNA content per cell and on generation time. By plotting the data on semilog paper (as log counts min^{-1} *vs* time), the apparent dependence of DNA synthesis kinetics on the number of cells per millilitre of culture is eliminated (Smith & O'Leary, 1968). Incorporated radioactivity becomes a direct measure of the amount of DNA present if a thymine auxotroph is used for radioactive thymine incorporation studies (Smith & O'Leary, 1968; Doudney,

1971). In prototropic strains the internal pool of nonradioactive precursors of DNA presumably competes with the incoming radioactive thymidine. The problem of the attainment of pool equilibrium of the specific precursor can be disregarded, even at short times after UV, if the cells are grown in media containing label for several generations before irradiation (Smith, 1969). Small changes in the DNA content of cells immediately after UV irradiation, however, will not be observable with even modestly prelabelled cultures (Setlow & Setlow, 1970) and the point which an investigator wishes to emphasize should determine the method of labelling eventually employed.

2.1.5 Effect of inhibitors on DNA synthesis in irradiated bacteria

A study of the effect of repair protein inhibitors on DNA synthesis in UV-irradiated cells can contribute greatly towards a better understanding of the DNA repair systems present and functioning in a specific organism.

Caffeine has long been known to affect repair processes in bacteria (Witkin, 1959). Investigators found that treatment of irradiated *E.coli* cells with sublethal concentrations of caffeine inhibits both LHR and HCR (Feiner & Hill, 1963; Sauerbier, 1964; Harm, 1966)

and causes a reduction in cell survival and UV resistance (Witkin, 1959; Rothman, 1980). Caffeine is a purine analogue which binds to single-stranded DNA and to irradiated double-stranded DNA (Domon *et al*, 1970), presumably at locally denatured areas adjacent to the dimers (Swenson, 1976; Rothman, 1980); caffeine does not bind to unirradiated, active DNA (Domon *et al*, 1970). The bound caffeine specifically inhibits excision repair processes in *E.coli* (Rupert & Harm, 1966; Setlow, 1967; Setlow & Carrier, 1968; Swenson & Carrier, 1968; Swenson, 1976; Fong & Bockrath, 1979; Rothman, 1980) by interfering with both the endonucleolytic cleavage at the dimer sites and the exonucleolytic digestion of the dimer-containing strand as it is displaced during resynthesis (Roulland-Dussoix, 1967; Harm, 1970; Rothman, 1980). Caffeine treatment consequently also results in a great reduction in the degradation of DNA observed in irradiated *recA*$^-$ *E.coli* cells (Shimada & Takagi, 1967; Yonei & Nozu, 1972), as well as in the degradation of irradiated phage lambda DNA (Roulland-Dussoix, 1967).

Several investigators agree that caffeine inhibits the incision step of excision repair (Shimada & Takagi, 1967; Fong & Bockrath, 1979; Rothman, 1980), but Fong & Bockrath (1979) claimed that is has little effect on the post-incision steps of excision repair. Rothman (1980) studied the effect of caffeine on irradiated *E.coli* cells in

detail and found that caffeine does not only inhibit the rate at which incisions are made, but also prolongs the time needed to rejoin the discontinuities. He showed, however, that although the initial rate of incision is inhibited, the final number of incision breaks and the repair patch size in UV-irradiated *E.coli* cells are unaffected by the postirradiation addition of caffeine. In addition, Rothman (1980) also observed that although sublethal concentrations of the inhibitor do not alter the colony-forming ability of unirradiated organisms (Fong & Bockrath, 1979), caffeine has a general deleterious effect on cell growth in unirradiated *E.coli* cells, as exemplified in a decrease in the rate of cell multiplication.

The effects of the protein inhibitor, chloramphenicol, on DNA synthesis and recovery of irradiated cells are varied and complex (Swenson, 1976). Chloramphenicol added at time zero after UV irradiation prevents the induced synthesis of any of the damage-inducible proteins: as such it inhibits reinitiation of DNA synthesis in irradiated cells (Harold & Ziporin, 1958; Doudney, 1959; Drakulić & Errera, 1959; Doudney, 1960 and 1968; Rudé & Doudney, 1973) and leads to a decrease in survival and a loss in the shoulder of survival curves in wild type and *uvr* mutants of *E.coli* B/r and K12 (Alper & Gillies, 1960; Okagaki, 1960; Bridges, 1972; Ganesan & Smith, 1972).

Youngs *et al* (1974) showed that chloramphenicol specifically interferes with the closing of incision breaks in irradiated *E.coli* wild type and *polA1* mutants but that it has little effect on break closure in *recA*, *recB* and *lexA* mutants. The inhibition by chloramphenicol of the induction of the $recA^+$ protein leads to enhanced degradation of irradiated DNA in wild type and $polA^-$ cells (Harold & Ziporin, 1958; Youngs *et al*, 1974). Harold & Ziporin (1958) added chloramphenicol at intermediate times after UV irradiation and showed that the amount of protein synthesized prior to chloramphenicol addition determined the rate of subsequent DNA synthesis.

Chloramphenicol does not interfere with excision repair in *E.coli* (Swenson & Setlow, 1966; Bridges, 1972). Short treatments with chloramphenicol can actually enhance the recovery of irradiated *E.coli* B cells (Gillies & Alper, 1959; Suzuki & Iwama, 1960; Drakulić *et al*, 1966; Morozov & Myasnik, 1970) by delaying initiation of DNA replication and so allowing more time for excision repair enzymes to excise dimers in advance of DNA replication (Swenson, 1976).

2.1.6 Effect of UV irradiation on viability and respiration in *E.coli*

When the effect of UV radiation on the growth of organisms is discussed, it is often not clear by what

criteria it has been judged. Cell growth can include an increase in cell size, cell number, or both and is therefore not necessarily the same process as cell division which only leads to an increase in cell number. DNA synthesis is a prerequisite for cell division (Swenson & Setlow, 1966), but is not sufficient to ensure an increase in viability. Viable cells are defined as cells able to form a colony on agar plates (Schenley *et al*, 1976a) and include only cells able to successfully complete repair of their damaged DNA (Ganesan, 1974). If the colony-forming ability of irradiated bacteria is studied, it is therefore preferable to refer to the effect of UV irradiation on the viability, rather than on the growth, of cells.

By monitoring the turbidity of irradiated cells in liquid suspension, it was observed that growth is delayed in UV-irradiated *E.coli* cells for some time following irradiation (Swenson, 1976). An increase in colony-forming units (c.f.u.), however, continued for another 20 minutes in irradiated *E.coli* cells under conditions where DNA synthesis was inhibited in essentially all cells (Helmstetter & Pierucci, 1968). This slight increase in viability immediately after UV irradiation has been observed by several investigators and probably only occurs in cells which have completed their rounds of DNA replication prior to UV irradiation (Barner & Cohen, 1956;

Okagaki,1960; Smith, 1969; Swenson & Schenley, 1970b). Smith (1969) reported that this increase in c.f.u. is rapid and continues for between 30 and 60 minutes in both radioresistant and radiosensitive *E.coli* cells. He thought that it may represent a unique repair mode, since generation times recorded for these irradiated cells were too long for the rapid increase to be due to growth.

The increase is followed by a plateau in viability, but a small drop in turbidity is often observed during this period of division delay due to the conversion of normal-sized cells to smaller ones (Swenson & Schenley, 1974b). This delay lasts for about three generation times in irradiated *E.coli* B/r cells during which time the DNA content of the cells increases three-fold (Smith, 1969). Cell division resumes after this lag period at a rate similar to that of the unirradiated control (Smith, 1969; Swenson & Schenley, 1970b; Boyle & Swenson, 1971).

Cell growth is closely linked to respiration in un-irradiated and UV- irradiated repair- proficient cells (Hamkalo & Swenson, 1969), except when protein and/or RNA synthesis are inhibited. Respiration in sublethally irradiated *E.coli* cells continues at a near-normal rate for the first 60 minutes after

irradiation and is then partially inhibited for a period of time dependent on the UV fluence and the growth conditions of the cells (Kelner, 1953; Hamkalo & Swenson, 1969; Swenson & Schenley, 1970a and 1970b). This inhibition lasted for several hours in *E.coli* cells irradiated to 1,0% survival (Swenson & Schenley, 1970a). The partial inhibition of growth and respiration in irradiated *E.coli* cells occurs at the same time as DNA synthesis is resumed and Hamkalo & Schenley (1969) suggested that this slowing down of the metabolism may correct the unbalanced growth that occurred during the period of DNA inhibition (Barner & Cohen, 1956). The respiration process is under DNA control (Kelner, 1953; Hamkalo & Schenley, 1969) and damage to the DNA is proposed to cause the $recA^{+}lexA^{+}$-dependent derepression of an operon under repressor control in unirradiated cells (Swenson & Schenley, 1972; Swenson & Schenley, 1974a). Excess protein is then produced which causes respiration to cease in the lethally damaged cells (Swenson, 1978). Those cells that stop respiring completely, do so irreversibly and are dead (Swenson & Schenley, 1974a; Schenley *et al*, 1976a). Any interference with protein and RNA synthesis during the 60 minute period following UV radiation prevents the induction of the regulator protein, and respiration and growth is maintained in these irradiated cells (Swenson & Schenley, 1970a). The increased DNA degradation which

occurs at high UV fluences presumably results in the loss of DNA control over synthesis and growth processes; growth in these heavily irradiated cells is inhibited, but respiration continues (Hamkalo & Schenley, 1969; Swenson & Schenley, 1974a; Swenson, 1976). The cessation of respiration and growth coincides with the selective loss into the suspending medium of unaltered pyridine nucleotides, NAD^+ and $NADP^+$ (Swenson & Schenley, 1970b; Swenson, 1978) and the rapid alteration of the cell envelopes of cells which have stopped respiring. The cell membranes of such cells become more permeable and eventually these cells lyse (Swenson & Schenley, 1974b). These changes in lethally damaged cells are often masked in rich media by the rapid growth and respiration of the surviving fraction.

2.1.7 Cell death after UV irradiation

Cell death is the most obvious consequence of UV damage to DNA, and yet the cause of radiation death remains poorly understood despite the extensive studies on this subject in *E.coli* (Swenson, 1978). One of the more popular theories put forward to explain radiation death includes the dysfunction of normal DNA replication (Hanawalt, 1966; Doudney, 1968; Billen, 1969; Billen & Carreira, 1971; Doudney, 1971;

Rudé & Doudney, 1973) due to deficiencies in the various DNA repair pathways (Boyle & Setlow, 1970), which eventually leads to the appearance of double-strand breaks in the damaged DNA (Moss & Davies, 1974; Bonura & Smith, 1975). A poor relationship has been found, however, not only between DNA synthesis and survival, but also between survival and DNA repair (Swenson, 1976 and 1978). DNA synthesis continues in cells which have lost the ability to form colonies on agar plates (Smith, 1969). Incomplete dimer excision seems to cause little permanent damage to cells. A small number of dimers are lethal to *uvr*$^-$ cells, while a greater number of unexcised dimers is tolerated by surviving *uvr*$^+$ cells (Sedliaková *et al*, 1977). Although there is a parallel between excision of dimers and increase in cell survival during LHR in *E.coli* B/r cells, the maximum extent of excision is only 24% (Tang & Patrick, 1977a). Schenley *et al* (1976b) found that the same number of pyrimidine dimers are excised from the living and the dead cells in irradiated cultures. Reirradiation of the surviving fraction causes immediate further excision of dimers, indicating that the excision system in the living cells is not inactive, but appears to ignore the remaining dimers (Schenley *et al*, 1976b). It is therefore possible that viable cells are the ones in which dimers have been

selectively removed, or accurately bypassed, at certain critical sites.

Another important physiological explanation for radiation death is the cessation of respiration in dead cells. It is still not clear whether the cessation of respiration observed in lethally damaged cells occurs because the cells are dead, or whether cells die because respiration ceases (Swenson, 1978). It appears, however, to be a *recA*$^+$-regulated process that only occurs in lethally damaged cells (Swenson & Schenley, 1974a). Swenson *et al* (1977 & 1978) demonstrated that cyclic 3',5'-adenosine monophosphate (cAMP) and its receptor protein regulate the cessation of respiration and cell killing in irradiated *E.coli* cells. The only known function of the cAMP-CRP complex is in the transcription of inducible operons in *E.coli* (Pastan & Adhya, 1976) and it has no known regulatory role in DNA repair and DNA replication.

This finding may be linked to the very interesting recent results of Petranović *et al* (1979) which indicate that death in *E.coli* after UV irradiation may be an active "self-destruct" program. They claimed that chromosomal inactivation and cell death are programmed into the *recA*$^+$-dependent (SOS) sequence of postirradiation events and suggested that this inactivation takes place in all cells that have not fully repaired their DNA. Conse-

quently Trgovčević *et al* (1981) found that *E.coli* AB 1157 cells with UV-damaged genomes cease to replicate their DNA following a well-defined time-point of postirradiation incubation. Initially DNA synthesis in these cells resumes at the same rate as in unirradiated control cells at all UV fluences lower than a certain critical dose. This concept of programmed death (Trgovčević *et al*, 1981) strengthens the idea of other investigators that the radiation death of organisms is the result of induced biochemical and physiological responses that become irreversible (Swenson, 1976 and 1978).

As there are several indications that far-UV-induced systems in the obligate anaerobe *B.fragilis* differ in certain aspects from those reported for *E.coli* (Jones *et al*, 1980; Jones & Woods, 1981; Slade *et al*, 1981), we investigated the effect of far -UV irradiation on macromolecular synthesis and viability in *B.fragilis* under anaerobic condtions. Since no *B.fragilis* UV mutants are available, these studies only represent the effect of UV irradiation on the kinetics of incorporation of radioactive precursors into DNA, RNA and protein of irradiated wild type *B.fragilis* cells.

2.2 MATERIALS AND METHODS

2.2.1 Bacteria and media

The specific strain of *B.fragilis*, Bf-2, used in this research was originally isolated from a clinical specimen taken from a patient at the King Edward VIII Hospital in Durban. It was identified as a *B.fragilis* strain by workers in the Department of Microbiology in the Faculty of Medicine at the University of Natal, Durban. The identity of the strain was confirmed by Dr L.V. Holdeman, V.P.I. & S.U., Anaerobe Laboratory, Blacksburg, USA. Bf-2 is a non-bacteriocin producer and is sensitive to most of the bacteriocins produced by other strains of *Bacteroides*. It is also sensitive to all the bacteriophages specific for the *Bacteroides* group.

The strain was maintained on brain heart infusion (BHI) slopes and beef liver anaerobic medium and was subcultured at 12-monthly intervals. For routine use, the cultures were maintained on BHI agar plates which were subcultured at weekly intervals and stored in anaerobic jars under 100% carbon dioxide.

These studies were carried out on the Bf-2 strain which was also utilized in previous studies on far-UV

irradiation (Jones *et al*, 1980; Jones & Woods, 1981; Slade *et al*, 1981; Slade *et al*, 1983a and 1983b). Brain heart infusion broth and agar, supplemented with hemin, menadione, and cysteine (Holdeman and Moore, 1972) were used for bacterial propagation at 37°C. Prereduced one-quarter-strength Ringer solution was used as a dilution buffer (Jones & Woods, 1981). Irradiation and radioactive labelling of the cells were carried out in a defined minimal medium (Varel & Bryant, 1974). The doubling time during exponential growth of *B.fragilis* in this medium was 62 min at 37°C. All manipulations were carried out under stringent anaerobic conditions in an anaerobic glove cabinet (Forma Scientific, Marietta, Ohio).

2.2.2 UV irradiation

Overnight cultures of *B.fragilis* cells in brain heart infusion broth were diluted 100-fold in minimal medium and reincubated until the cultures reached a turbidity of 0,2 at 600 nm (1×10^8 to 2×10^8 c.f.u. ml^{-1}). Samples (9ml) of the cultures were irradiated in open glass petri dishes (90mm in diameter) with a Fluotest Piccolo Hanau Quartz germicidal lamp which emitted the majority of its output at 254 nm. The dose rate was measured with a Blak-Ray UV meter (model J-225; UV Products Inc, San Gabriel, Calif.) and samples were irradiated at a dose rate of 1,0 J $m^{-2}s^{-1}$.

Survival curves of bacteria irradiated with increasing fluences were determined, and labelling experiments were carried out with cultures which were irradiated to different survival levels.

2.2.3 DNA, RNA and protein synthesis

DNA synthesis was determined by the incorporation of [2-^{14}C]thymidine (4 μCi ml^{-1}) or [*methyl*-^{3}H] thymidine (10 μCi ml^{-1}), RNA synthesis by incorporation of [5,6-^{3}H] uracil (15 μCi ml^{-1}), and protein synthesis by the incorporation of [^{35}S] methionine (20 μCi ml^{-1}) into cold trichloroacetic acid (TCA) precipitable cellular material. The labelled chemicals were supplied by The Radiochemical Centre, Amersham, England and the final concentrations of thymidine, uracil and methionine were 7, 15 and 25 μg ml^{-1} respectively, which were saturating for both uptake and incorporation over 240 min.

The effect of UV irradiation of DNA, RNA and protein synthesis was determined in prelabelled and unlabelled cultures (Smith & O'Leary, 1968; Setlow & Setlow, 1970). In order to prelabel cells overnight brain heart infusion broth cultures were inoculated into minimal medium and incubated until the cultures reached a turbidity of 0,15 at 600 nm. The label was added and

the cultures reincubated until a turbidity of 0,2 at 600 nm was reached before the cultures were irradiated with UV light (prelabelled cells). In experiments involving unlabelled cells, the label was added to the culture immediately after irradiation. For dual labelling experiments, the cells were pre-labelled for 60 min with [^{14}C] thymidine. The labelled cells were then harvested by centrifugation, resuspended in fresh minimal medium without label and incubated for a further 50 min before irradiation and the addition of [^{3}H] thymidine.

2.2.4 Effect of chloramphenicol and caffeine on DNA synthesis

The effect of chloramphenicol (5 μg ml^{-1}) and caffeine (1 mg ml^{-1}) on DNA synthesis after UV irradiation was determined with prelabelled and unlabelled cells. The inhibitors were not present during irradiation, but were added to minimal medium cultures immediately after irradiation. The minimal inhibitory concentrations of chloramphenicol and caffeine were 1 μg ml^{-1} and 2,5 mg ml^{-1}, respectively.

2.2.5 Effect of UV on viability

Exponential phase cells were irradiated in minimal medium with 0, 30, 40, 50 and 70 J m^{-2} and reincubated

at 37°C under anaerobic conditions. Viability was determined after different time intervals by plating on brain heart infusion agar. Each sample was plated onto a minimum of three agar plates and the colonies were counted after 48 h at 37°C under anaerobic condition.

2.3 RESULTS

2.3.1 Effect of UV irradiation on DNA synthesis

UV irradiation of *B.fragilis* cells prelabelled with [^{14}C]thymidine resulted in an initial rapid net loss of label from the TCA precipitable fraction (Fig 2.1). The DNA degradation phase was followed after approximately 60 min by the net synthesis of DNA. Dual labelling experiments were carried out to determine whether DNA synthesis was masked during the DNA degradation phase (Fig 2.2). DNA synthesis was decreased after UV irradiation, but never totally inhibited. This DNA synthesis was masked in the prelabelling experiments by the phase of extensive DNA degradation immediately after exposure to UV irradiation. Degradation of prelabelled DNA continued for 40 min postirradiation before reaching a plateau between 40 and 160 min when there was no net loss of [^{14}C]thymidine from the DNA.

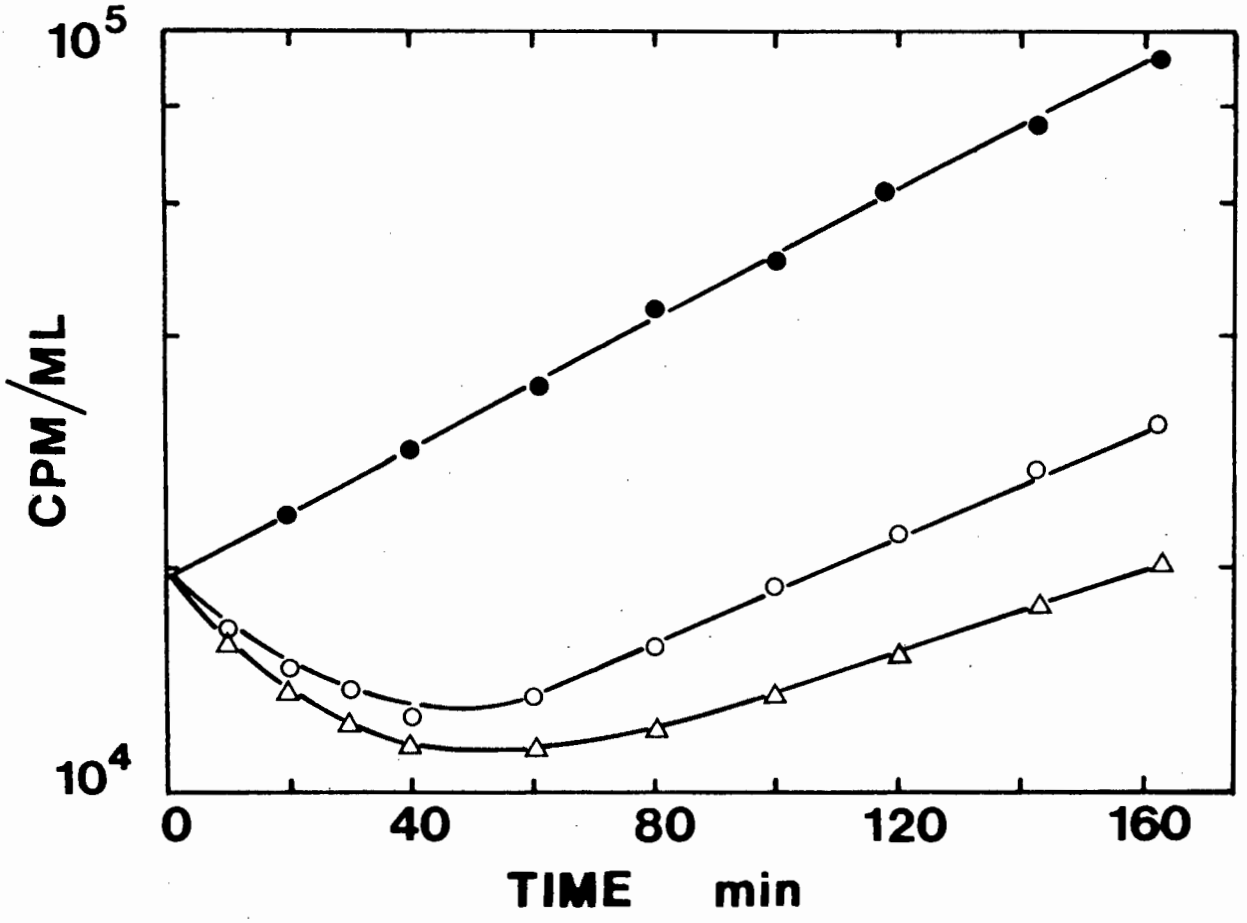

FIG 2.1 Effect of UV irradiation on DNA synthesis in prelabelled *B.fragilis* cells. The [^{14}C] thymidine was added to the cells 60 min prior to irradiation. The cells were irradiated and reincubated in the presence of the label. Unirradiated control (●); cells irradiated with 50 J m^{-2} (11% survival) (○) and 70 J m^{-2} (1,2% survival) (△).

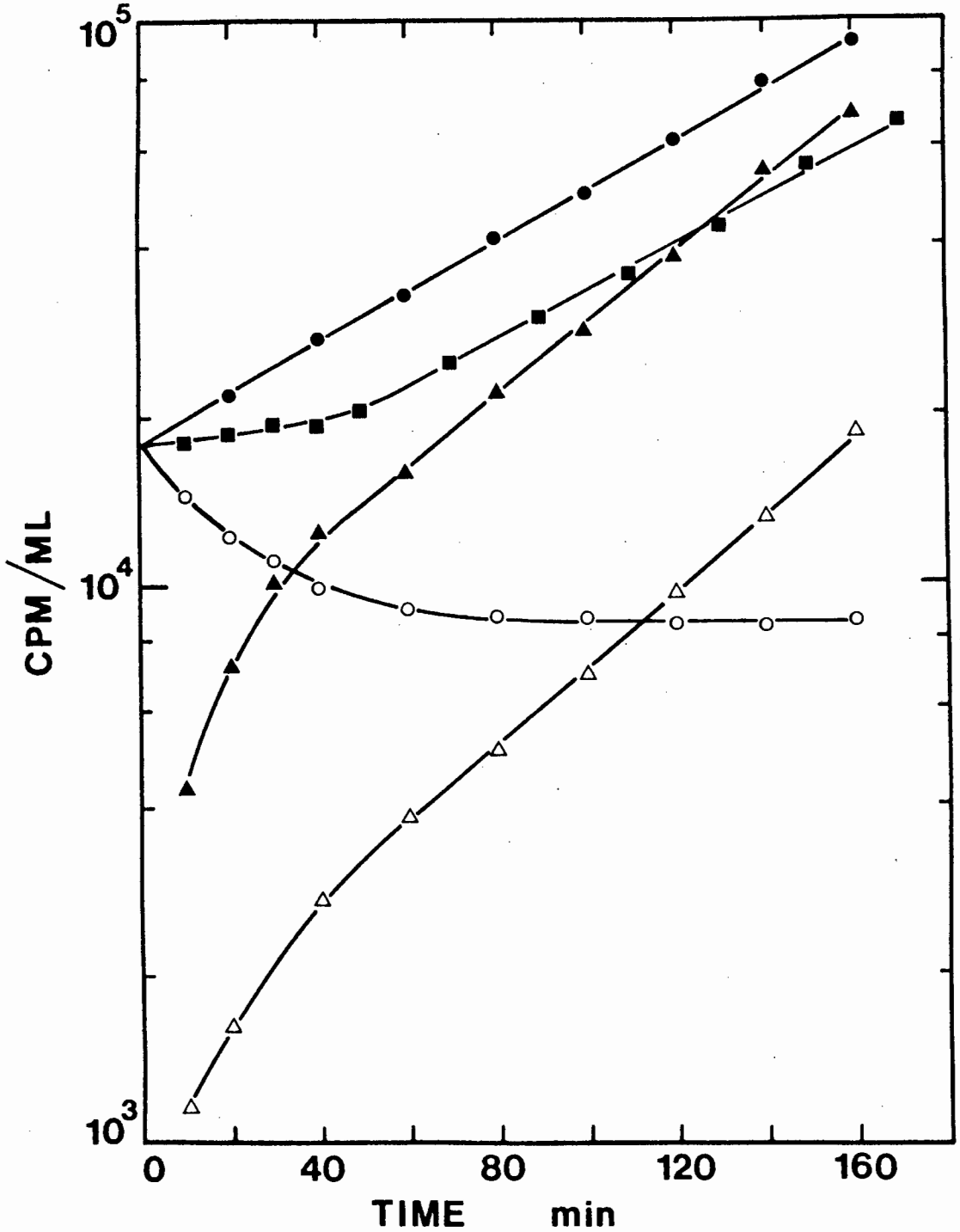

FIG 2.2 Effect of UV irradiation on DNA synthesis in dual-labelled *B.fragilis* cells. Cells prelabelled with [^{14}C] thymidine for 60 min were harvested by centrifugation, resuspended in fresh medium without [^{14}C] thymidine and incubated for a further 50 min before UV irradiation (50 J m^{-2}, 11% survival) and the addition of [^{3}H] thymidine (time 0 min). Unirradiated prelabelled control reincubated in [^{14}C] thymidine immediately after harvesting (■) and at time 0 min (●). Irradiated cells with (△) and without (○) [^{3}H] thymidine added at the time of irradiation (time 0 min). Unirradiated cells with [^{3}H] thymidine added at time 0 (▲).

In order to carry out the dual labelling experiments it was essential to harvest the cells by low speed centrifugation. Although all the manipulations were carried out under stringent anaerobic conditions the unirradiated cells were affected by the centrifugation procedure and required a further 50 min incubation before DNA synthesis was resumed (Fig 2.2). In all duel labelling experiments the cells were irradiated at least 50 min after centrifugation.

DNA synthesis was also determined in unlabelled cultures which were irradiated with increasing doses of UV light prior to the addition of [^{14}C] thymidine. In these experiments DNA synthesis was measured by the net accumulation of label into TCA precipitable material over a fixed period of time. In unirradiated unlabelled *B.fragilis* cells exponential incorporation of [^{14}C] thymidine similar to that in unirradiated prelabelled cells was observed 30 min after the addition of the label, which indicated that pool equilibrium of the thymidine precursor in the prototrophic *B.fragilis* cells was attained within that 30-min period. DNA synthesis was reduced after UV irradiation and showed linear kinetics for a dose-dependent period (Fig 2.3). At higher UV doses there was a longer period of linear DNA synthesis. DNA synthesis during this linear phase also decreased

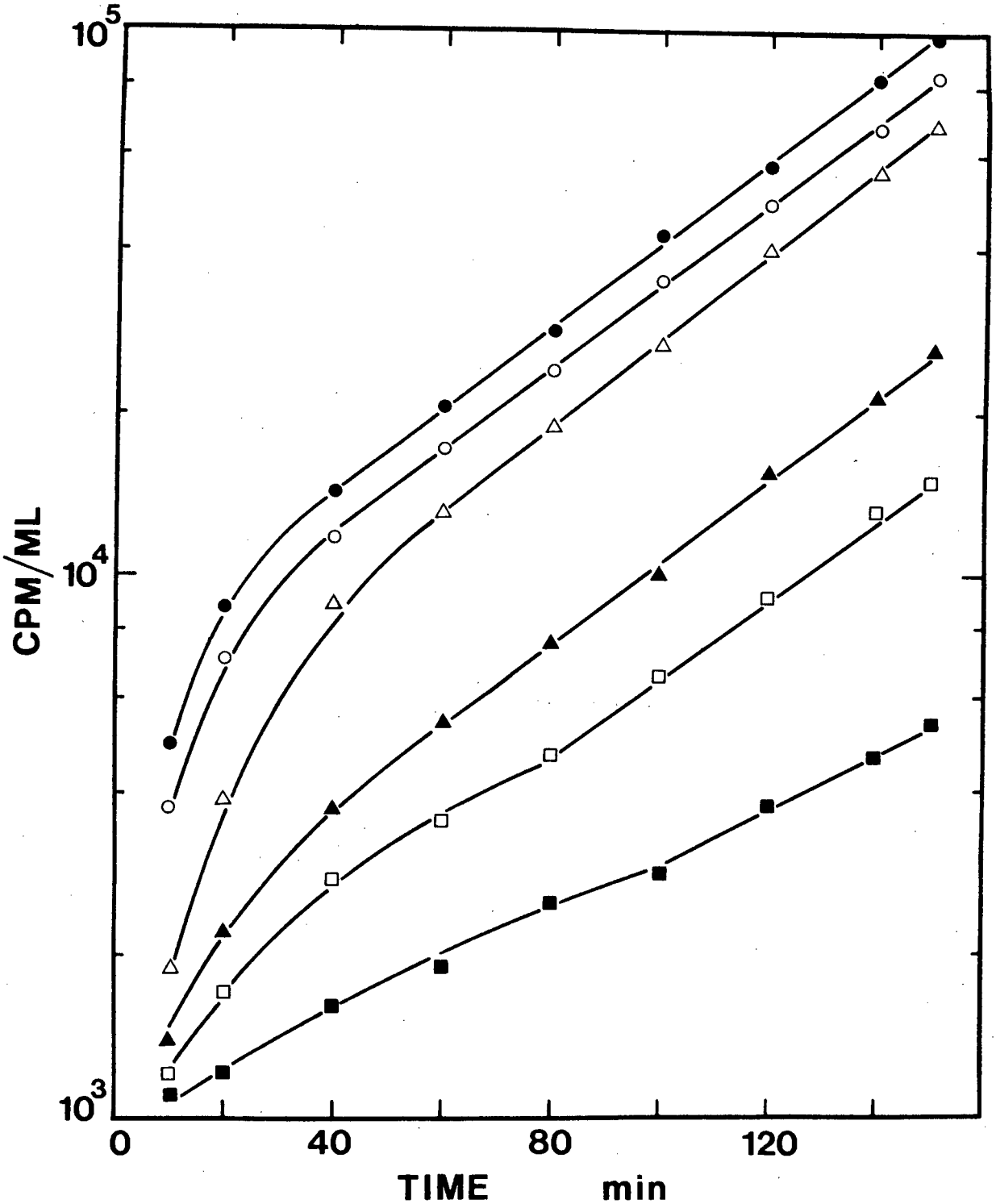

FIG 2.3 Effect of increasing doses of UV irradiation on DNA synthesis in unlabelled *B.fragilis* cells. Exponential phase cells were irradiated and labelled with [^{14}C] thymidine. Unirradiated control (●). Cells irradiated with UV (J m^{-2}): 20 (91% survival) (○), 30 (62% survival) (△), 40 (26% survival) (▲), 50 (11% survival) (□) and 70 (1,2% survival) (■).

with increasing UV doses. After this period of depressed linear synthesis, DNA synthesis increased in an exponential fashion in all irradiated cultures. At the high UV doses (26, 11 and 1,2% survival) the rate of DNA synthesis after the linear phase varied between 30 cpm ml^{-1} min^{-1} at 1,2% survival and 155 cpm ml^{-1} min^{-1} at 26% survival. The rate of DNA synthesis at 86 and 62% survival was 300 and 295 cpm ml^{-1} min^{-1}, respectively, whereas the rate in the unirradiated control was 305 cpm ml^{-1} min^{-1}. The new exponential rates of synthesis persisted until the end of the experiment (150 min).

2.3.2 Effect of chloramphenical and caffeine on DNA synthesis after UV irradiation

Experiment with prelabelled cells indicated that extensive degradation of DNA was inhibited by the addition of chloramphenicol immediately after UV irradiation (Fig 2.4). In these prelabelled cells net DNA synthesis was detected 100 min after UV irradiation. Experiments with unlabelled cells showed that DNA synthesis after UV irradiation was markedly reduced by chloramphenicol but not inhibited completely. After 100 min DNA synthesis in chloramphenicol-treated irradiated cells resumed at the same exponential rate as irradiated cells without chloramphenicol. DNA synthesis in unirradiated cells was

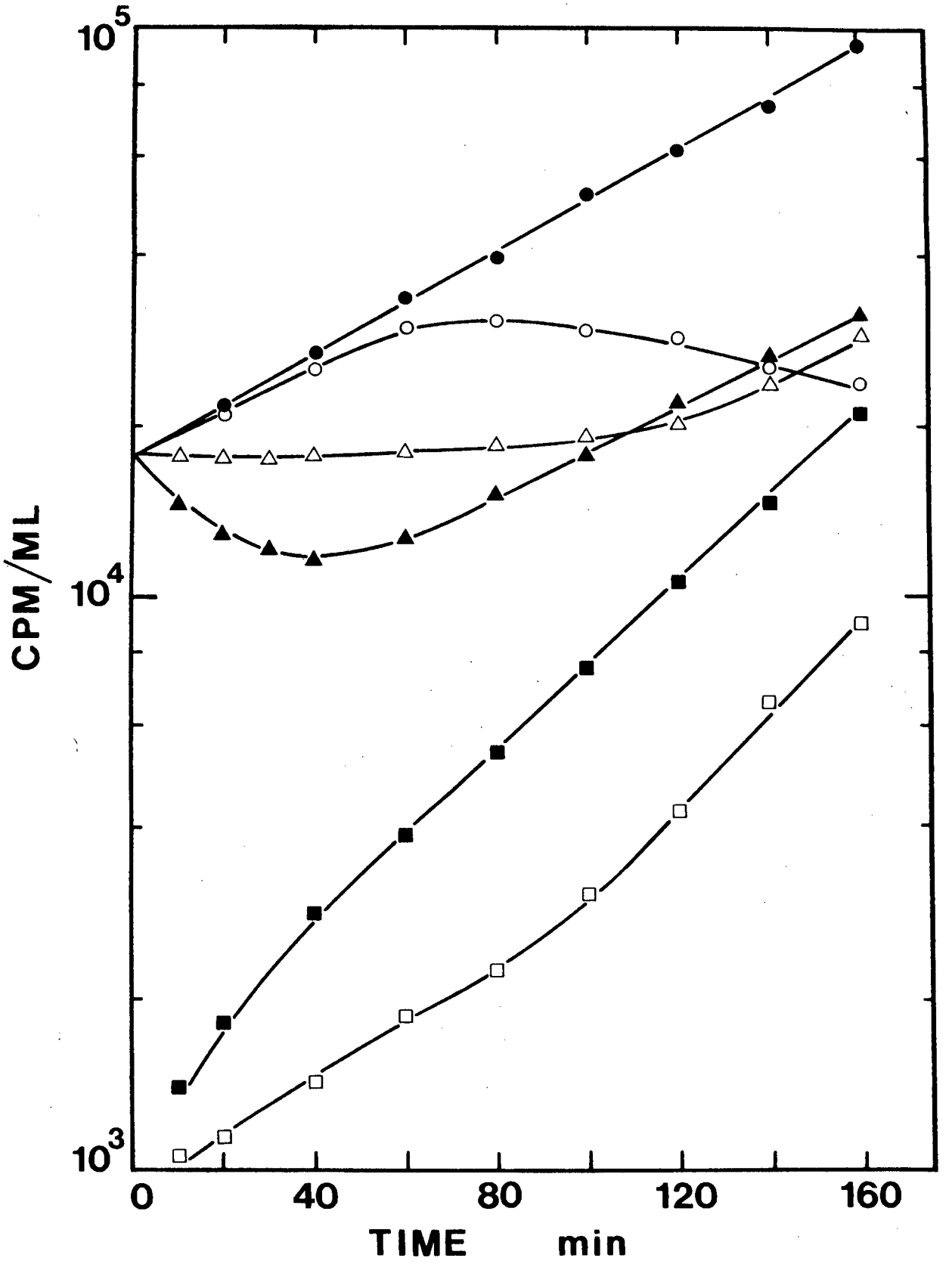

FIG 2.4 Effect of chloramphenicol on DNA synthesis after UV irradiation. Cells prelabelled with [^{14}C] thymidine prior to UV irradiation with (△) and without (▲) chloramphenicol. Cells irradiated and then labelled with [^{14}C] thymidine, with (□) and without (■) chloramphenicol. Unirradiated cells prelabelled with [^{14}C] thymidine with (○) and without (●) chloramphenicol. Cells were irradiated with 50 J m^{-2} UV (11% survival).

decreased by chloramphenicol and totally inhibited after 60 min. Chloramphenicol (5 μg ml^{-1}) inhibited protein synthesis immediately in unirradiated cells (Fig 2.6).

Caffeine treatment of prelabelled irradiated cells inhibited extensive DNA degradation immediately and DNA synthesis 40 min after UV irradiation (Fig 2.5). DNA synthesis in unlabelled UV irradiated cells was completely inhibited by caffeine 40 min after irradiation. Caffeine did not affect DNA synthesis in unirradiated cells.

2.3.3 Effect of UV irradiation on RNA and protein synthesis

UV irradiation of *B.fragilis* cells resulted in a dose-dependent decrease in RNA and protein synthesis (Fig 2.6). Protein synthesis was less sensitive to UV irradiation than RNA synthesis; 60 min after irradiation at 50 J m^{-2} protein and RNA synthesis were reduced by 28% and 42%, respectively, compared with the unirradiated control.

After irradiation (50 J m^{-2}, 11% survival) RNA synthesis increased linearly for approximately 60 min before it resumed exponentially at a somewhat lower rate than in the unirradiated control. At the same dose protein

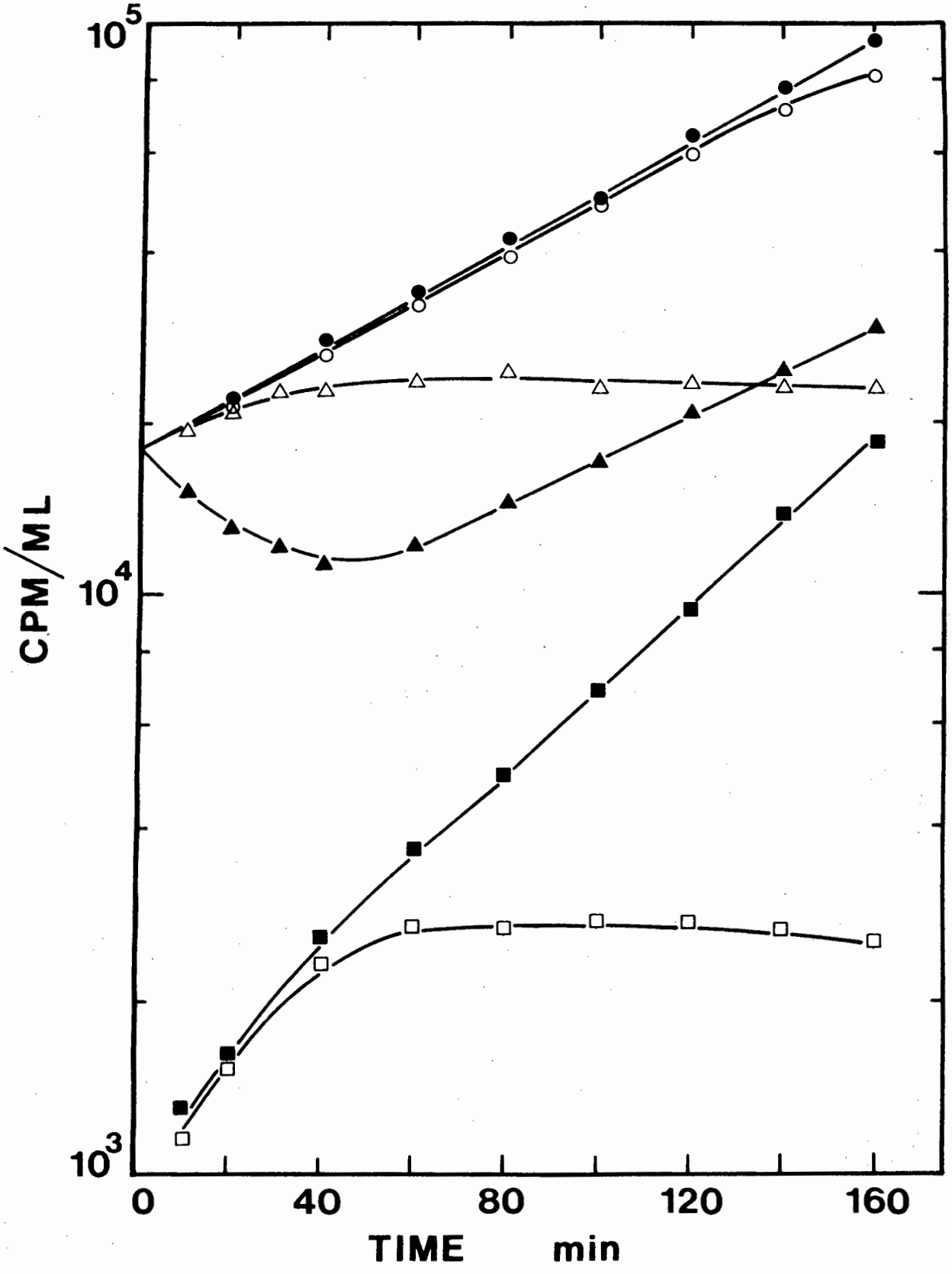

FIG 2.5 Effect of caffeine on DNA synthesis after UV irradiation. Cells prelabelled with [^{14}C] thymidine prior to UV irradiation with (△) and without (▲) caffeine. Cells irradiated and then labelled with [^{14}C] thymidine with (□) and without (■) caffeine. Unirradiated cells prelabelled with [^{14}C] thymidine with (○) and without (●) caffeine. Cells were irradiated with 50 J m^{-2} UV (11% survival).

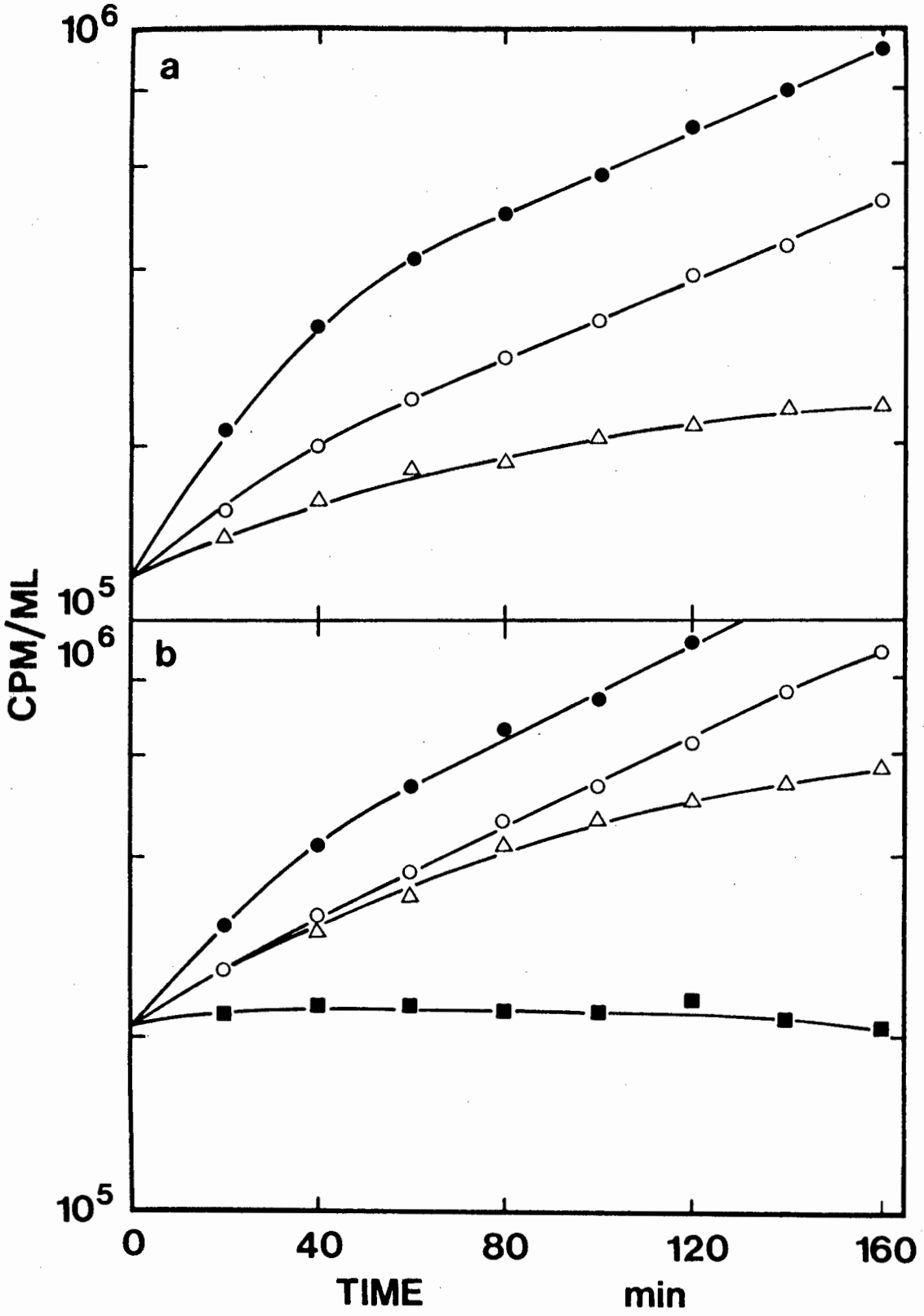

FIG 2.6 Effect of UV irradiation on RNA (a) and protein (b) synthesis. Cells prelabelled with [^{3}H] uracil (a) and irradiated with UV fluences of 50 (11% survival) (○) and 70 J m^{-2} (1,2% survival) (△). Cells prelabelled with [^{35}S] methionine (b) and irradiated with UV fluences of 50 (11% survival) (○) and 70 J m^{-2} (1,2% survival) (△). Unirradiated control (●). Unirradiated cells prelabelled with [^{35}S] methionine and treated with chloramphenicol (5 μg ml^{-1}) at time 0 (■).

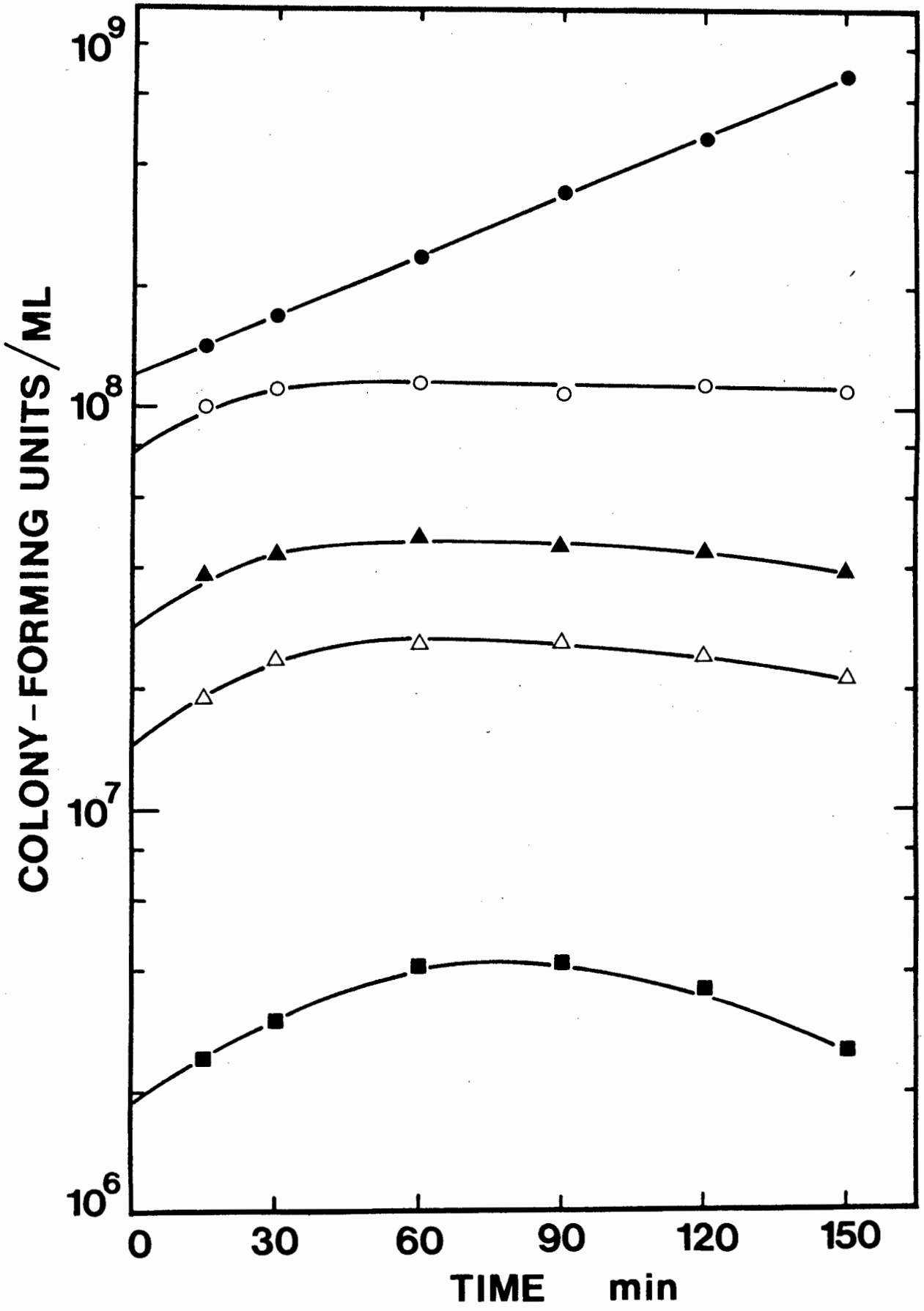

FIG 2.7 Effect of UV irradiation on colony formation by *B.fragilis* cells under anaerobic conditions. Cells were irradiated with increasing UV fluences and samples were plated onto brain heart infusion agar at time intervals after irradiation. UV fluences ($J\ m^{-2}$): 30 (62% survival) (●); 40 (24% survival) (○); 50 (12% survival) (▲) and 70 (1,5% survival) (△). Unirradiated control (■).

synthesis continued in an exponential fashion. At 1.2% survival both RNA and protein synthesis were markedly reduced and showed linear kinetics until the end of the experiment (160 min).

2.3.4 Effect of UV irradiation on colony formation

The ability of *B.fragilis* to form colonies on agar medium was not affected immediately by UV irradiation (Fig 2.7). The c.f.u. continued to increase in number for a dose-dependent period before colony formation was inhibited. Inhibition of colony formation occurred sooner in cells irradiated with lower doses of UV light than in cells irradiated with high UV doses. The division delay persisted until the end of the experiment (2,5h). In cultures irradiated to 1,5% survival there was a decrease in viability after colony formation was inhibited.

2.4 DISCUSSION

Experiments with the anaerobe *B.fragilis* on the effect of UV irradiation on DNA synthesis support the conclusion of Setlow & Setlow (1970) that there is no unique best method for investigating macromolecular synthesis in irradiated bacteria. Rapid degradation of DNA immediately after UV irradiation of *B.fragilis* cells

was only detected with prelabelled cells. This extensive degradation masked the discovery, which was made with unlabelled cells, that DNA synthesis decreased but was never totally inhibited by UV irradiation and continued during the degradation phase.

The characteristics of DNA synthesis in *B.fragilis* cells irradiated with far-UV light under anaerobic conditions differ from those reported for *E.coli* where DNA synthesis is stopped completely and immediately for a dose-dependent period by UV irradiation (Kelner, 1953; Hanawalt & Setlow, 1960; Setlow *et al*, 1963; reviewed by Swenson, 1976, and Hall & Mount, 1981). Trgovčević *et al* (1980) reported that this inhibition of DNA synthesis is dependent on a functional *recA* gene product. The discovery that UV radiation under anaerobic conditions did not inhibit DNA synthesis may suggest that *B.fragilis* cells do not have an *E.coli recA*-type system or that a protein with a similar recombination function does not have a similar regulatory role in *B.fragilis*. However, although the synthesis of DNA in *B.fragilis* was not inhibited after UV irradiation, the kinetics of synthesis changed to a linear mode for a dose-dependent period, before exponential DNA synthesis resumed. Smith (1969) reported that there was no abrupt cessation of DNA synthesis in *E.coli* B cells after UV irradiation.

Another difference between the postirradiation DNA kinetics of *B.fragilis* and that of *E.coli* involved the degradation of irradiated DNA. Although some DNA degradation does occur in wild type *E.coli* cells after UV irradiation, it is limited (Swenson, 1976). Extensive "reckless" DNA degradation occurs in *recA*$^-$ mutants following UV irradiation (Clark, 1973). A similar extensive degradation of DNA after UV irradiation was observed in wild type *B.fragilis* cells. In *E.coli* the *recA*$^+$ protein binds to single-stranded regions on the chromosome and so inhibits the endonuclease and exonuclease activities of the *recBC* DNase on single-stranded DNA which account for 75 to 90% of the DNA degradation in irradiated cells (Marsden *et al*, 1974; Satta *et al*, 1979; Williams *et al*, 1981).

Other *E.coli* repair-deficient mutants also degrade DNA excessively after UV irradiation and include *uvrD* (Ogawa *et al*, 1968), *polA1* (Boyle & Setlow, 1970), *resA1* (also deficient for DNA polymerase 1)(Kato & Kondo, 1970) and *lexA* (Howard-Flanders & Boyce, 1966; Howard-Flanders & Theriot, 1966).

The extensive degradation by *E.coli recA* cells does not seem to be the cause of death because multiple mutants, such as *recA recB recC* have about the same radiation sensitivity as *recA*, but degradation of DNA is greatly reduced (Willetts & Clark, 1969). Studies by Pollard and Snipe (1977) revealed that inhibition of postirradiation DNA degradation is also absent in *Pseudomonas* BAL-31, of marine origin.

DNA degradation in *E.coli recA* mutants is greatly reduced by the addition of caffeine immediately after UV irradiation (Shimada & Takagi, 1967; Yonei & Nozu, 1972). Caffeine also inhibited DNA degradation in irradiated *B.fragilis* cells. Degradation of DNA in irradiated *E.coli* cells is supposedly initiated at single-strand breaks in the DNA produced by the enzymes involved in the excision of damage (Howard-Flanders & Boyce, 1966). Caffeine was previously shown to cause a marked decrease in the number of c.f.u. after UV irradiation of *B.fragilis* cells (Jones *et al*, 1980) and it is thus suggested that the degradation of DNA is an important part of repair processes in irradiated cells of this organism.

The inhibition by chloramphenicol of the extensive DNA degradation that occurred after UV irradiation in *B.fragilis* suggests that protein synthesis is required

for degradation. In *E.coli* excision occurs in the presence of chloramphenicol (Swenson & Setlow, 1966). Chloramphenicol reduced DNA synthesis in irradiated *B.fragilis* cells but did not inhibit it completely. Although chloramphenicol prevents the resumption of DNA synthesis in irradiated *E.coli* (Swenson, 1976), Swenson & Setlow (1966) reported that in a radiation-resistant *E.coli* B strain DNA synthesis is resumed at the same time in irradiated cells with and without chloramphenicol. The DNA synthesis in irradiated *B.fragilis* cells treated immediately with chloramphenicol is analogous to stable DNA replication in *E.coli* in that it is an abnormal, UV-induced DNA synthesis which can occur in the absence of protein synthesis (Kogoma & Lark, 1975; Lark & Lark, 1978). However, *B.fragilis* differs from *E.coli* in that in *E.coli* protein synthesis is necessary during a 40-min period after irradiation before the addition of chloramphenicol for the initiation of stable DNA synthesis (Kogoma *et al*, 1979).

The finding that RNA and protein synthesis are, in that order, less sensitive to UV than DNA synthesis, is in accord with the current views of macromolecular synthesis in living organisms. RNA synthesis in irradiated *B.fragilis* cells was linear during the phase of reduced linear DNA synthesis, while in *E.coli*

linear synthesis is observed for both RNA and protein during the time that DNA synthesis is inhibited (Hanawalt & Setlow, 1960; Swenson & Setlow, 1966).

In *B.fragilis* colony formation was not inhibited immediately by UV irradiation and there was an inverse relationship between UV dose and inhibition of viability. An initial transient increase in colony formation after UV irradiation has also been reported in *E.coli* under conditions where DNA synthesis was inhibited in virtually all the cells (Okagaki, 1960; Helmstetter & Pierucci, 1968; Smith, 1969; Swenson & Schenley, 1970b). Smith (1969) observed that with both UV-sensitive and -resistant *E.coli* mutants there was an inverse relationship between UV dose and inhibition of colony formation. After the period of division delay, cell division resumes in irradiated *E.coli* cells at a rate similar to that of the unirradiated control (Smith, 1969; Swenson & Schenley, 1970b; Boyle & Swenson, 1971). Cell division in irradiated *B.fragilis* cultures did not resume during the time in which the experiment was performed (2,5h).

The drop in viability observed in *B.fragilis* cells irradiated to 1,5% survival is as yet unexplained. It occurred under conditions where DNA synthesis continued. In irradiated *E.coli* cells, however, DNA

synthesis continues for many generations in cells which are unable to form colonies on agar plates (Smith, 1969) and there is generally a poor relationship between DNA synthesis and survival of irradiated cells (Swenson, 1978). A drop in the number of c.f.u. in an irradiated *E.coli* culture is caused by the lysis of lethally damaged cells in which respiration has ceased (Swenson & Schenley, 1974b).

The subject of DNA repair and recombination in *E.coli* has moved well beyond the point we have reached in our studies on macromolecular synthesis in irradiated *B.fragilis* cells. The advanced knowledge of DNA repair in *E.coli* is largely due to the availability of specific *E.coli* UV mutants altered in one or more of the established repair pathways. At present investigations in *B.fragilis* are hampered by our inability to isolate UV-sensitive or -resistant mutants in spite of an extensive screening programme. Our results on the characteristics of DNA synthesis after UV irradiation and the difficulty experienced in isolating mutants in general in *B.fragilis* suggest that the UV repair and recombination systems in this anaerobe may differ from those in *E.coli*, which tend to be accepted as typical for bacteria in general. The basic studies on *B.fragilis* reported in this chapter may provide a starting point for future work on UV repair and recombination in this important anaerobe.

CHAPTER 3

UV LIGHT INDUCTION OF PROTEINS IN *BACTEROIDES FRAGILIS* UNDER ANAEROBIC CONDITIONS

3. Introduction

Exposure of *E.coli* cells to UV irradiation and other agents which damage the DNA, or interfere with its replication, induces a complex co-ordinated set of survival-promoting responses collectively known as the SOS functions (Radman, 1975; Witkin, 1976; Gottesman, 1981). The SOS response involves many physiological changes including the appearance of increased repair and mutational activities, inhibition of cell septation, induction of certain prophages and the aberrant reinitiation of DNA replication at the chromosome origin after synthesis arrest (Witkin, 1976; Hall & Mount, 1981). The expression of all these changes is co-ordinately regulated by the *recA* and the *lexA* gene products. DNA damage activates the protease activity of the basal $recA^+$ protein to cleave the *lexA* gene product and other cellular repressors of SOS functions, so switching on the expression of the entire SOS event (Little & Harper, 1979; Brent & Ptashne, 1980; Craig & Roberts, 1980; Little *et al*, 1980). Kenyon & Walker (1980) showed that DNA

damage in *E.coli* induces increased transcription at several specific loci on the chromosome and many investigators reported that *de novo* protein synthesis is required for the expression of the pleiotropic SOS response (Witkin, 1976; Eisenstark, 1977). The *din* (*d*amage-*in*ducible) genes whose expression is stimulated by DNA damage, or inhibition of DNA synthesis, and whose products have been associated with the occurrence of particular SOS processes, include the *recA* (Inouye & Pardee, 1970; Gudas & Pardee, 1975; Little & Hanawalt, 1977; West & Emmerson, 1977) *lexA* (Little & Harper, 1979; Brent & Ptashne, 1980) *umuC* (Bagg *et al*, 1981), *uvrA* (Kenyon & Walker, 1980, 1981) *uvrB* (Fogliano & Schendel,1981; Kenyon & Walker, 1981), *sfiA* (Huisman & D'Ari, 1981) and the *himA* (Miller *et al*, 1981) genes.

No report has been published as yet on the possible stimulation of protein production by far-UV radiation in obligate anaerobic bacteria.

3.1.1 The inducing signal

The induction of several SOS events and proteins by DNA damage is a well studied event, but little

is known about the initial molecular event or signal which triggers the expression of SOS functions. Prophage induction and the enhanced synthesis of the *recA*$^{+}$ protein are the best understood responses to DNA damage and these phenomena have consequently been used by molecular investigators as measures of successful repressor inactivation following DNA damage.

Although the entire SOS response is induced by sudden stops in the DNA replication, a stalled replication fork *per se* is not sufficient, or necessary, to induce *recA* expression in *E.coli* (Casaregola *et al*, 1982a). Both Monk & Gross (1971) and Schuster *et al* (1973) showed that prophage lambda was also not induced when the DNA replication was arrested at the end of a cycle in *E.coli* initiation defective *dnaA* (Ts) mutants at the non-permissive temperature. Neither could naladixic acid induce the synthesis of *recA* protein in a *recB*$^{-}$*recC*$^{-}$ strain even though the DNA synthesis was inhibited (Gudas & Pardee, 1975). UV ligh and bleomycin, on the other hand, could induce the *recA*$^{+}$ protein in a *dnaA* mutant at the restrictive temperature (Gudas & Pardee, 1976) and Gudas & Pardee proposed in 1975 that the inducing signal for *recA*$^{+}$ protein induction is

most probably a product of DNA degradation. In support of this proposal it has been found that nalidixic acid (Gudas, 1976), but not novobiocin (Smith & Oishi, 1978) can induce *recA*$^{+}$protein production. Novobiocin inhibits DNA replication by inactivating gyrase subunit B without concomitant DNA degradation, while treatment with nalidixic acid causes inhibition of DNA synthesis due to the inactivation of gyrase subunit A (Higgins *et al*, 1978), followed by the selective degradation of nascent DNA.

If the progress of the replication fork is blocked during a replication cycle, induction of SOS functions is generally observed (Casaregola *et al*, 1982a). Under these conditions localised degradation of newly synthesized DNA occurs near the replication fork due to the action of the *recBC*-coded exonuclease V (Lieberman & Oishi, 1974). This degradation produces single-stranded regions (gaps) in the chromosome (MacKay & Linn, 1976; Oishi & Smith, 1978; Taylor & Smith, 1980) and many investigators claimed that it is the gaps in the chromosome which serve as SOS inducing signals (Sedgwick *et al*, 1978; Sussman *et al*, 1978; Bockrath & Hanawalt 1980). A functional *recBC* gene product is essential for the induction of prophage after DNA damaging

treatments such as precursor deprivation, DNA inhibition by nalidixic acid, a temperature shift-up of a *dna* (Ts) elongation mutant, and treatment with micrococcal and pancreatic DNases (that only nick DNA) (Gudas & Pardee, 1976; Irbe & Oishi, 1980; Oishi *et al*, 1981). Exonuclease V-mediated degradation is, however, not the only source of suitable signal fragments and although a *recB* mutation considerably delays the induction of prophage and $recA^+$ protein in *E.coli*, UV light, bleomycin and mitomycin C can still induce these events in the absence of a functional *recBC* DNase (Gudas & Pardee, 1975; Little & Hanawalt, 1977; Oishi *et al*, 1981). The results of Oishi *et al* (1978) strongly suggested that induction by UV light and mitomycin C involves DNA degradation by multiple DNases, only one of which is the $recBC^+$ DNase. McPartland *et al* (1980) found, for example, that the product of the $recF^+$ gene (exonuclease VIII) is more important than the $recBC^+$ DNase for the generation or transduction of a signal for SOS induction following UV damage to the DNA. It is unclear whether the signals produced by the alternate pathways ($recBC^+$- and $recF^+$-dependent) are the same (Little & Mount, 1982).

There is no casual relationship between the produc-

tion of DNA fragments and *recA*$^+$ induction (Little & Hanawalt, 1977) and degradation *per se* is not sufficient to provide a signal (Baluch *et al*, 1980). The extensive degradation which occurs in plasmolyzed *E.coli* cells following treatment with pancreatic DNase does not cause the induction of ø80 prophage and induction is only observed in the presence of a functional *recBC* DNase (Oishi *et al*, 1981). Little & Hanawalt (1977) thought that the inducer must be a specific DNA fragment with a unique structure, not unlike the fragments produced by exonuclease V activity.

Oishi and co-workers (1981) studied the ability of various oligonucleotides with specific structures (resulting from DNA digestion) to inactivate the ø80 phage repressor and to relieve the requirement for exonuclease V activity. They found that specific dinucleotides with the sequence d(R-G), where R is a purine nucleoside, have strong prophage inducing activities. It is essential that the sugar moieties of the active dinucleotide must be a deoxyribose and that it has quanine at the 3' end; r(A-G), r(G-G) and d(G-I) were found to have no inducing activities. Both d(A-G) and d(G-G) induce prophage nearly as effectively as UV irradiation. There is also another class of oligodeoxyguanylates

which has prophage inducing activity: a deoxyguanosine oligonucleotide with a chain length between 6 and 18 can effectively induce prophage as long as it has a phosphate at the 5' position (Oishi *et al*, 1981). This is in agreement with the results of Craig & Roberts (1980) who found that proteolysis of lambda repressor requires, besides ATP, a polynucleotide chain of about 16 to 20 nucleotides. Induction triggered by d(G-G) is independent of the *recBC* gene product and no degradation of chromosomal DNA occurs following d(G-G) treatment. Oishi *et al* (1981) therefore speculated that d(G-G) may be equivalent to the *in vivo* signal produced by $recBC^+$ DNase activity on predegradative DNA, or to an intermediate product in a $recBC^+$-independent SOS pathway. The results of Oishi and co-workers, however, also reveal a high degree of complexity and specificity in the early steps of SOS regulation: ø80 prophage is induced by d(AGGp), but this oligonucleotide cannot induce $recA^+$, or promote cleavage of lambda repressor *in vivo* (Oishi *et al*, 1981).

The concentration of nucleoside triphosphate present in the cell affects the $recA^+$ cleavage acticity; the cleavage of $lexA^+$ protein has been shown to specifically require ATP or an analog, ATP-γ-S (Horii *et al*, 1981a). The increase in dATP levels

observed during some SOS-inducing treatments (such as nalidixic acid treatment and thymine starvation) (Neuhard & Thomassen, 1971) is therefore an important component of the SOS-inducing signal and Oishi & Smith (1978) reported that dNTPs can trigger ø80 prophage induction in the presence of a functional *recBC* DNase.

3.1.2 Mechanism of activation

The initial event leading to the derepression of all SOS operons is the activation of the protease activity of the *recA*$^+$ protein (Craig & Roberts, 1980 and 1981; Phizicky & Roberts, 1981). The reaction requires Mg^{2+}, ATP and a polynucleotide cofactor, and the formation of a complex between the *recA*$^+$ protein, single-stranded DNA and nucleoside triphosphate seems to be the critical event in this activation (Craig & Roberts, 1981; Phizicky & Roberts, 1981; Flory & Radding, 1982). Phizicky & Roberts (1981) proposed that the conformational change (Devoret, 1981) that occurs in *recA*$^+$ protein during formation of the *recA*$^+$-DNA-nucleoside triphosphate complex exposes a site that can bind and cleave repressor protein. Damage to cellular DNA promotes this reaction by providing single-stranded regions for the binding of the *recA*$^+$ protein. The cell concentration of the activated form of *recA*$^+$ protein

depends on how many sites on the DNA are available to activate the protein (Little *et al*, 1980), and there is an optimum ratio of single-stranded DNA to *recA*$^+$ protease (about four nucleotides per *recA*$^+$ monomer); excess single-stranded DNA inhibits this reaction (Little & Mount, 1982). The active *recA*$^+$ protease can then derepress the SOS operons by the specific cleavage of the bacterial *lexA*$^+$ protein (Little *et al*, 1981) and the prophage repressors (Roberts *et al*, 1978).

Tif mutants exhibit constitutive repressor cleavage, and thus constitutive high levels of expression of SOS functions, in the absence of DNA damage. The *tif* protein is more basic than the wild type *recA*$^+$ protein (Gudas & Mount, 1977) and McEntee & Weinstock (1981) suggested that the *tif* mutation alters the binding sites of the *recA*$^+$ protein so that the *tif(recA)* protein is activated by complex formation with short single-stranded regions or gaps that normally occur near the growing fork of replicating chromosomes (McEntee & Weinstock, 1981; Phizicky & Roberts, 1981). These single-stranded regions are too short for the activation of the normal *recA*$^+$ enzyme. *Tif* expression is suppressed in *dnaA* mutants at 42^{o}C when the chromosome is fully replicated and no new replication forks are formed (D'Ari *et al*, 1979).

3.1.3 Kinetics of induction and decay of SOS repair activities

Genes involved in the SOS response differ in their sensitivity to inducing treatment (Huisman & D'Ari, 1981) and the various SOS functions in *E.coli* are expressed in the same damage-induced population with quite different kinetics (Devoret, 1981; Pollard *et al*, 1981). The induction of the *recA*$^+$ protein reaches a maximum after 60 to 90 minutes after UV irradiation (Salles & Paoletti, 1983), while the average time required for other SOS functions to reach a maximum following the same inducing treatment, is approximately 30 minutes (Witkin, 1975; Defais *et al*,1976). The time required for repressor inactivation by proteolytic cleavage is specific for each type of cellular repressor. The *lexA*$^+$ protein is cleaved within 3 minutes after a mild UV treatment (Little & Mount, 1982), but lambda repressor inactivation is a slow cellular process that takes a generation time to reach completion (Gudas & Pardee, 1975; Bailone *et al*, 1979): one mole of *recA*$^+$ protein cleaves 0,41 mole of lambda repressor in one hour in the presence of ATP and polynucleotides (Craig & Roberts, 1980; Phizicky & Roberts, 1981). Resnick & Sussman (1982), however, found that

specific concentrations of cobalt increase the *in vitro* rate of lambda repressor cleavage and the rate of strand assimilation by the $recA^+$ protein.

Kenyon & Walker (1980) thought that the different lag times required for the expression of the *din* genes indicated different triggers for activation of these operons. Oishi *et al* (1979) suggested that all the different altered DNA structures resulting from the different DNA damaging treatments are converted to a common final predegradative structure, which is then susceptible to degradation by specific DNases, mainly $recBC^+$ DNase. There is a definite correlation between the timing of DNA degradation and inactivation of repressor following the inducing treatment (Oishi *et al*, 1981). DNA damaging agents that cause strand breaks, such as bleomycin and streptonigrin, cause immediate degradation of DNA and exhibit inactivation of repressor molecules as early as 10 to 15 minutes after treatment. Treatment with agents that modify DNA bases, such as UV irradiation and mitomycin C which cause pyrimidine dimer formation and alkylation, respectively, causes relatively slower DNA degradation after a short lag period of approximately 20

minutes. Maximum inactivation of repressor by these agents occurs after about 30 minutes. The efficiency of UV reactivation of phage lambda, for example, reached a peak after 30 minutes of incubation of the UV-irradiated host before infection with the irradiated phage (Defais *et al*, 1976). The inhibition of DNA synthesis by thymine deprivation, or temperature shiftup of *dna* (Ts) elongation mutants, leads to the slow induction of prophage after DNA degradation at the very late stage of 60 to 90 minutes after initiation of the treatment (Oishi *et al*, 1981). The DNA structure resulting from these last two treatments exhibits no DNA damage other than an immobilized replication fork and is comparatively resistant to degradative attacks by DNases.

The SOS operons are only transcribed while SOS-inducing conditions prevail and their products are rapidly degraded in the cell (Witkin, 1976). Both enhanced Weigle reactivation and the ability to enhance UV mutagenesis decayed with a half-life of 30 minutes once the optimum level was achieved, and these two SOS phenomena were no longer detectable after 90 minutes under non-inducing conditions (Witkins, 1975; Defais *et al*, 1976). Salles & Paoletti (1983) found that the presence of residual

unexcised dimers does not permanently induce the formation of *recA*$^+$ protein. The rate of synthesis of *recA*$^+$ protein declines between 30 to 60 minutes (Little & Hanawalt, 1977), and Little *et al* (1981) suggested that the rate at which the *recA*$^+$ protein decays after UV irradiation is primarily controlled by the rate at which dimers are excised.

Casaregola *et al* (1982a) reported a rapid turnoff of *recA* expression once DNA replication is restored (within 10 minutes of restoration), presumably because the signal molecule is rapidly consumed in the DNA polymerization reaction. The creation and disappearance of the signal molecule is therefore proposed to be ultimately reponsible for turning the SOS functions on and off (Casaregola *et al* 1982a). Witkin (1976) reported that four hours after SOS inducing treatments all the signal molecules are removed and the wild type *E.coli* cells have returned to equilibrium.

3.1.4 The damage-inducible gene products

3.1.4.1 *RecA* gene and protein

Induction of the SOS response in *E.coli* is

accompanied by the appearance of a prominent 37 000 to 40 000-molecular weight protein, the *recA*$^+$ protein, formerly described as protein X (Inouye, 1971; Gudas & Pardee, 1975; McEntee, 1976; Emmerson & West, 1977; Gudas & Mount, 1977; Little & Kleid, 1977; McEntee & Epstein, 1977). The basal level of *recA*$^+$ protein represents about 0,02% of the total protein produced (Casaregola *et al*, 1982b). When *E.coli* cells are subjected to DNA damaging treatments, transcription of the *recA* gene increases considerably (McPartland *et al*, 1980) and Casaregola *et al* (1982b) reported a 17-fold increase within 10 minutes after UV irradiation or thymine starvation. Salles & Paoletti (1983) found that the fully-induced level of the *recA*$^+$ protein represents a 20- to 55-fold amplification of the basal level, and this may amount to as much as 3% of the total cell protein synthesized (Gudas, 1976). *RecA*$^+$ protein production reaches its peak values at indentical times of 60 to 90 minutes, whatever the initial UV dose (Salles & Paoletti, 1983). Ninety percent of the total *recA*$^+$ protein is found in the cytoplasm and 10% in the cell membrane (Gudas & Pardee, 1976).

Cloning of the *recA* gene (Clarke & Carbon, 1976; McEntee, 1976; McEntee & Epstein, 1977; Ogawa *et al*, 1979; Sancar & Rupp, 1979; Uhlin & Clark, 1981) has

greatly facilitated the purification and identification of the *recA* gene product (Shibata *et al*, 1979; Weinstock *et al*,1979; MacKay *et al*, 1980; Sancar *et al*, 1980; Cox, 1981; Shibata *et al*, 1981).The operon region was identified as the *rnm* region (Volkert *et al*, 1981) and *rnmA* and *rnmC* mutants fail to amplify *recA* protein after SOS-inducing treatments (Volkert *et al*, 1979). The *recA* gene was sequenced by Horii *et al* (1980) and Sancar *et al* (1980). The coding region of the *recA* gene has 1059 base pairs (bp) which specify a 352-amino acid protein with alanine and phenylalanine as its NH_2- and COOH-terminal amino acids respectively. The NH_2-terminal half contains all three cysteines and both histidines, amino acids that are part of the active site of most proteases (Sancar *et al*, 1980).

All repressor-operator interactions studied so far involved DNA dyad symmetry (Sancar *et al*, 1981b), and the *lexA* binding site in the *recA* gene also contains a perfect dyad symmetry with the exception of two bp in the centre (Sancar *et al*, 1982). In addition, the *recA* gene contains three translational symmetries with characteristic direct repetition of 8 to 11 bases (Sancar *et al*, 1980). These sequences have invert repeats at the ends which are flanked by direct repeats, features possessed by known IS elements (Reed *et al*, 1979). These "pseudo -ISs" (Sancar *et al*, 1980)

are too small to code for proteins that promote transposition and Sancar *et al* (1980) speculated that these sequences played a role in the evolution of the *recA* gene, and perhaps of the *E.coli* chromosome itself.

The soluble $recA^+$ protein is isolated as a heteropolymer with a molecular weight of 150 000 after mild detergent lysis of host cells (McEntee, 1976). Oligomer formation depends on the ionic strength of the suspending solution and on protein concentration: low concentration of salt and high concentrations of protein favour aggregation (Ogawa *et al*, 1979). $RecA^+$ protein also aggregates in response to ATP, and the tetrameric form of $recA^+$ protein acts as an ATPase dependent on single-stranded DNA (Ogawa *et al*, 1979 ; Howard-Flanders, *et al*, 1981).

The highly pleiotropic $recA^+$ protein is an ATP-dependent DNA-binding protein which appears to have two distinct domains of activity in the *E.coli* cell (Morand *et al*, 1977), inactivated by two clusters of mutations, *lexB* and *recA* proper. The one domain is active in general recombination, recombinational repair and control of $recBC^+$ DNase-mediated degradation, while the other domain is involved in the

proteolytic regulation of SOS induction (Devoret, 1981). Amplification of the *recA*$^+$ protein leads to enhanced recombinational repair and consequently to increased survival of irradiated cells (Witkin, 1976). Induced *recA*$^+$ production is also required for the important non-recombinational function of inhibition of excessive DNA degradation by exonuclease V (Chapter 2). At the same time, high amounts of *recA*$^+$ protein exerts a negative control on its own expression because of its inhibition of the *recBC*$^+$-mediated generation of appropriate signals for induction of the *recA*$^+$protein (Volkert *et al*, 1981).

Genetic recombination promoted by *recA*$^+$ protein in wild type *E.coli* cells appears to occur by several pathways of which two have been well defined. The *recBC*$^+$-mediated pathway is constitutive and is catalyzed by the basal levels of *recA*$^+$ protein present in the cell, and is therefore *lexA*$^+$-independent (Clark, 1980). The *recF*$^+$-dependent pathway comes into operation once the *recBC*$^+$ pathway is blocked by mutations in the *recB*, *recC* or *sbc* genes. This pathway is *lexA*-dependent and inducible, but recombination proceeds more slowly than the *recBC*$^+$-dependent recombination (Clark, 1980; Bresler *et al*, 1981). The product of the

lexC gene, the single-strand binding protein (SSB)(Meyer *et al*, 1979; Sancar & Rupp, 1979; Meyer *et al*, 1980) augments the stoichiometric interaction of the *recA*$^+$ protein with single-stranded DNA and promotes the recombinational activities of the *recA*$^+$ protein (Flory & Radding, 1982). Baluch *et al* (1980) suggested that the SSB protein is required for the stabilization and maintenance of DNA gaps after UV irradiation. Salles & Paoletti (1983) reported that the *uvrA*$^+$ protein exerts a negative control on the *recA*$^+$ protein activity because it effectively competes with *recA*$^+$ protein for binding to damanged DNA (Clark, 1980).

A comparative study of the induction of *recA*$^+$ protein and the concomitant occurrence of the other SOS events (Pollard *et al*, 1981) revealed the interesting finding that the amplification of *recA*$^+$ protein is not a prerequisite for the initiation of some SOS events (Devoret, 1981). The low constitutive levels of *recA*$^+$ protein in uninduced *E. coli* cells are sufficient, given the appropriate inducing signal to activate the *recA*$^+$ protease, to derepress the entire SOS event (Bailone *et al*, 1979; Devoret, 1981). Devoret (1981) showed that both Weigle reactivation and prophage induction are fully inducible under conditions preventing the

amplification of *recA*$^+$ protein.

The derepressed promotor of the *recA* gene is one of the strongest in *E.coli* (Sancar *et al*, 1980; Salles & Paoletti, 1983). The induced rate of synthesis of the *recA*$^+$ protein (10 molecules per second for high UV doses) approaches the rate of the product of the *tufA* gene, one of two constituents of EF-Tu which is known to have the highest rate of synthesis in *E.coli* (equal to 34 molecules per second) (Pedersen *et al*, 1978). The promotor sequence of the *recA* gene displays many similarities to that of the strongest early promotors of phage T5.

3.1.4.2 *LexA* gene and protein

All the inducible SOS functions in *E.coli* seem to be under direct or indirect control of the *lexA*$^+$ protein, which in turn is subject to derepression by the activated *recA*$^+$ protein. The *lexA* gene has been cloned and the *lexA*$^+$ protein purified to near homogenicity by Horii *et al* (1981a and 1981b), Markham *et al* (1981) and Miki *et al* (1981). The molecular weight of this repressor protein was found to be 22 000 to 25 000 (Little & Harper, 1979; Brent & Ptashne, 1980; Little *et al*, 1980).

Nucleotide sequencing of the *lexA* gene revealed a regulatory region, followed by a translational open reading frame, which encodes for a polypeptide of 202 amino acids (Horii *et al*, 1981b ; Markham *et al*, 1981). The $lexA^+$ protein is catalytically cleaved into two polypeptides by the $recA^+$ protein in the presence of ATP and single-stranded DNA. This unique cleavage site is between Ala^{84} and Gly^{85} near the centre of the $lexA^+$ protein (Horii *et al*, 1981a). The amino acid sequences around the cleavage site of the $lexA^+$ protein show strong homology with the cleavage sites of phage repressors (Horii *et al*, 1981a and 1981b) which are also cleaved into two fragments by activated *recA* protease (Roberts *et al*, 1979; Phizicky & Roberts, 1980). Horii *et al* (1981a) suggested that $recA^+$ protease recognises specific amino acid residues and/or some secondary structure around the cleavage site, and that this may play an essential role in the co-ordinated expression of the SOS responses.

The regulatory region of transcription initiation of the *lexA* gene (Horii *et al*, 1981a and 1981b; Miki *et al*, 1981) shows considerable homology to that of the *recA* gene (Horii *et al*, 1980; Sancar *et al*, 1980; Little *et al*, 1981) and the colicin E1 gene (Ebina *et al*, 1981). These homologous regions

represent the *lexA* repressor binding sites. Each site comprises of 20 bp and is known as an "SOS box" and contains numerous inverted repeat sequences (Little *et al*, 1981; Miki *et al*, 1981). The "SOS boxes" include the common sequences CTG and CAG separated by 10 bases. These "boxes" have also been found in the regulatory regions of the *uvrA* and *uvrB* genes (Sancar & Rupp, 1982) and could explain their repression by the *lexA* gene product and their SOS damage-inducibility. The *lexA* gene has two adjacent "SOS boxes" (Little *et al*, 1981) and exhibits negative autoregulation (Brent & Ptashne, 1981). The $lexA^+$ repressor appears to operate by excluding RNA polymerase from the promotor of the repressed gene (Little *et al*, 1981). The $lexA^+$ protein has less affinity for the *lexA* operator than for the *recA* operator (Brent & Ptashne, 1981), suggesting that as the $lexA^+$protein level builds up, the *recA* gene would be repressed faster than the *lexA* gene, ensuring proper repressor regulation of SOS functions when normal growth resumes.

3.1.4.3 *UvrA* and *uvrB* genes and proteins

The uvr^+-dependent excision repair is the most important system in *E.coli* for repairing pyrimidine dimers and other bulky DNA lesions causing major

helix distortions (Hanawalt *et al*, 1979; Lindahl, 1982). This ATP-dependent repair activity is catalyzed by the combined action of the products of the *uvrA*, *uvrB* and *uvrC* genes (Seeberg *et al*, 1976; Seeberg, 1978; Hanawalt *et al*, 1979), and the different subunits of the uvr^+ endonuclease show no detectable endonucleolytic activity by themselves (Lindahl, 1982). The products of the *uvr* genes have previously been regarded as being constitutively expressed since these enzymes can be easily detected in induced cells (Braun *et al*, 1976; Seeberg, 1978) and $uvrA^+$ $uvrB^+$-dependent excision repair is observed both in cells in which protein synthesis has been inhibited (Boyle & Setlow, 1970) and in $recA^-$ and $lexA^-$ cells (Clark & Volkert, 1978). However, recent reports indicate that both the *uvrA* gene (Kenyon & Walker, 1981) and the *uvrB* gene (Fogliano & Schendel, 1981) are in fact inducible by DNA damage in a $recA^+$ $lexA^+$-dependent fashion. The different *uvr* genes have also been cloned on small multicopy plasmids and the proteins purified to apparent homogenicity (Sancar *et al*, 1979, 1981a and 1981b; Yoakum & Grossman, 1981).

The *uvrA* gene codes for a monomeric protein of molecular weight 114 000 (Sancar *et al*, 1981b). The active $uvrA^+$protein is a DNA-binding protein

with ATPase activity which shows preferential affinity for UV-irradiated (single-stranded) DNA (Seeberg, 1978; Kacinski *et al*, 1981). The high affinity DNA-binding complex formed between *uvrA*$^+$ protein and ATP binds to the damaged region and then interacts with the *uvrB*$^+$ and *uvrC*$^+$ proteins to catalyze chain cleavage and excision of dimers (Witkin, 1976; Seeberg, 1981; Lindahl, 1982). The size of the helical region destabilized by *uvrA*$^+$ protein binding determines the patch size of the region subsequently excised (Lindahl, 1982).

The *uvrB* gene product can be induced by UV irradiation (Fogliano & Schendel, 1981) and nalidixic acid, but shows very little mitomycin C inducibility (Kenyon & Walker, 1981). Sancar *et al* (1981a) identified the *uvrB*$^+$ gene product as a polypeptide of 84 000 molecular weight, which directly interacts with the *uvrA*$^+$ protein and so binds tightly to single-stranded DNA, even though by itself the protein does not bind to DNA (Kacinski & Rupp, 1981). Sequencing of the regulatory region of the *uvrB* operon has revealed three promotor sequences within this DNA segment (Sancar *et al*, 1982). There is a *lexA* binding site in the P2 promotor region and both P2 and P3 operons are *lexA*$^+$-repressed due to the stalling of a transcribing RNA polymerase when it

reaches this repressor-operator complex. Derepression of the *lexA*$^+$-controlled *uvrB* operons is first detected five minutes after UV exposure and the induced rate of synthesis of the *uvrB* gene product can reach five times the basal rate (Schendel *et al*, 1982).

The P1 promotor is *lexA*$^+$-independent and its significance has not yet been determined (Sancar *et al*, 1982). This *lexA*$^+$-independent promotor appears to be relatively weak since Schendel *et al* (1982) showed that the *lexA*$^+$ protein can block up to 85% of the *uvrB* gene transcription. Sancar *et al* (1981a), however, reported a ratio of *uvrA*$^+$ protein to *uvrB*$^+$ protein in *recA*$^-$ cells of 1:7 and the constitutive expression of the *lexA*$^+$-independent operon may account for the production of this relatively high amount of *uvrB*$^+$ protein (Sancar *et al*, 1982). The constitutive production of the protein may be required for other cellular processes besides excision repair (Lindahl, 1982) since a *polA uvrB* mutant is nonviable while a viable *polA uvrA* mutant has been constructed (Shizuya & Dykhuizen, 1972; Morimyo & Shimazu, 1976). Mutations in the *uvrA* and *uvrC* genes do not significantly alter the synthesis of the *uvrB*$^+$ protein, either before or after UV exposure (Schendel *et al*, 1982).

Taking into consideration the *recA*$^+$ *lexA*$^+$dependent

inducibility of both *uvrA*$^+$ and *uvrB*$^+$ genes, Schendel *et al* (1982) suggested that the *uvrC* operon may also be inducible, although no evidence for this has yet been found. Yoakum & Grossman (1981) reported that the molecular weight of the *uvrC* gene product is 68 000, while Seeberg (1981) found that the expression of the cloned *uvrC* gene leads to the production of two polypeptides, one of molecular weight 30 000 and another of molecular weight 70 000.

From *E.coli* a protein with molecular weight of approximately 12 000 is isolated which is produced in normal amounts in *uvrC* mutants but appears to be missing from *uvrA* and *uvrB* mutants. This protein is referred to as *uvrA,B* endonuclease and may be inducible (Braun & Grossman, 1974; Grossman *et al*, 1975; Braun *et al*, 1976). The *uvrA,B*$^+$ endonuclease is ATP-independent, is active in the presence of EDTA, binds to irradiated DNA and although it is believed to act on many diaduct forms of damage, it preferentially releases dimers from UV-irradiated DNA in conjunction with exonuclease VII (Braun *et al*, 1976). The relationship between the small ATP-independent endonuclease and the *uvrABC*$^+$ complex is still unknown (Hanawalt *et al*, 1979).

The excision of dimers is not an appropriate criterion upon which to judge whether the *uvrA*$^+$ and *uvrB*$^+$ operons are induced in an organism or not, since these gene products only produce incisions at dimer sites (Seeberg *et al*, 1976; Seeberg, 1978). The rate-limiting dimer excision step depends on gene products such as the *uvrC*$^+$ protein and DNA polymerase 1 protein (Cooper & Hanawalt, 1972b; Glickman, 1974; Seeberg & Strike, 1976) which as yet have not been shown to be inducible (Ward & Murray, 1980).

3.1.4.4 *UmuC* gene and protein

Mutagenesis in *E.coli* is not a passive process but involves the active induction of an error-prone repair system in this organism following DNA damage. The 3'→5' exonuclease activity associated with procaryotic DNA polymerases has a proof-reading function which excises incorrectly incorporated 3'-terminal nucleotides. Villani *et al* (1978) postulated that DNA damage causes the induction of an inhibitor of the error-correcting exonucleolytic activity, which allows DNA replication to proceed past pyrimidine dimers at the expense of lower DNA replication fidelity (Devoret, 1981). The inducible *umuC* gene product (Bagg *et al*, 1981) seems to be the most likely candidate to act as the inhibitor of the copy-editing

mechanism in *E.coli* (Walker, 1978; Steinborn, 1979; Walker & Dobson, 1979). The *umuC* or *uvm* mutation causes *recA*$^+$*lexA*$^+$ bacteria to be non-mutable by UV irradiation and other DNA damaging agents, slightly UV-sensitive and deficient in their ability to carry out Weigle reactivation of irradiated phage. The plasmid pKM 101 containing the *muc* region (*m*utagenesis, *UV*, *c*hemical) (Mortelmans & Stocker, 1979) can suppress the *umuC* mutation and *umuC* mutant cells carrying this plasmid show enhanced mutagenesis following DNA damage.

The *umuC* gene of *E.coli* has recently been cloned and codes for two proteins of molecular weights 16 000 (*umuD*$^+$) and 45 000 (*umuC*$^+$), which are the same as the molecular weights reported for the *mucA*$^+$ and *mucB*$^+$ proteins, respectively (Walker & Dobson, 1979; Perry & Walker, 1982). The *mucC* gene is under direct repressor control of the *lexA* gene product and thus requires the protease activity of the *recA*$^+$ protein at the time of *umuC*$^+$ induction (Bagg *et al*, 1981).

3.1.4.5 *SfiA* gene and protein

Cell division in *E.coli* is tightly coupled to DNA replication. Unscheduled stops in DNA replication

result in a rapid arrest of cell division (septation) and increased filamentation (Witkin, 1976). The division inhibition is part of the *recA*$^{+}$*lexA*$^{+}$-regulated SOS event (Howe & Mount, 1978 and 1979) and is due to the rapid induction of a cell division inhibitor, the *sfiA* gene product, following even minor perturbations of DNA replication (George *et al*, 1975; Huisman *et al*, 198Oa;Huisman & D'Ari, 1981; D'Ari & Huisman, 1982; Quillardet *et al*, 1982). The *sfiA* operon is the most sensitive of the SOS operons to damage-inducing treatments and is used in a very sensitive chromotest for genotoxicity (Quillardet *et al*, 1982). Most genotoxins are inducers of the SOS response in bacteria and a *sfiA:lacZ* operon fusion in the tester strains gives a colorimetric quantitation of the amount of DNA damage done by the genotoxins. Mizusawa & Gottesman (1983) identified a *lexA* binding site ("SOS box") in the *sfiA* gene, which shares 18 to 20 bases of homology with the *lexA* binding site of the *recA* gene, and Huisman &.D'Ari (1983) showed that the *lexA* protein is the exclusive regulator of *sfiA* gene expression.

The target of the division-inhibiting *sfiA* gene product is the *sfiB* gene (George *et al*, 1975; Gottesman *et al*, 1981) situated in a cluster of genes involved

in envelope growth (Huisman *et al*, 1980b). Several investigators found that the *sfiB* gene product is indispensable for normal cell growth and suggested that the *sfiB* gene may code for an essential division function (Huisman *et al*, 1980b; Gottesman *et al*, 1981; Mizusawa & Gottesman, 1983).

Lon (deg, cap R) mutants filament extensively after DNA damage and also never recover the ability to continue normal cell division (Howard-Flanders *et al*, 1964). The complex phenotype of *lon* mutants includes the loss of the ATP-dependent protease La (Deg) activity (Chung & Goldberg, 1981), responsible for the degradation of certain abnormal proteins or protein fragments in the growing cell (Bukhari & Zipser, 1973; Gottesman & Zipser, 1978). George *et al* (1975) proposed that this protease normally rids the cell of the division-inhibiting $sfiA^+$ protein once normal DNA replication resumes, and this was very recently proved to be correct (Mizusawa & Gottesman, 1983). The $sfiA^+$ system is not involved in the regulation of cell division during normal (unperturbed) growth and Huisman *et al* (1983) suggested that the primary function of the *sfiA* gene product is to prevent untimely division and the formation of DNA-less cells once DNA replication has been perturbed. The *sfiA* and *sfiB* mutations do not affect the other SOS

repair functions (Huisman *et al*, 1980b). The *sulA* and *sulB* genes are allelic to the *sfiA* and *sfiB* genes (Gottesman *et al*, 1981) since their phenotypes are indistinguishable and they map in the same region of the chromosome (Johnson & Greenberg, 1975; Johnson, 1977; Huisman *et al*, 1980b). Mizusawa & Gottesman (1983) cloned the *sfiA(sulA)* gene and identified an 18 kilodalton polypeptide as the product of the *sfiA* gene.

3.1.4.6 *HimA* gene and protein

Whereas the $recA^{+}$protein is a major component of general recombination pathways in *E.coli*, site-specific recombination in this organism is dependent on the action of an 11 000-molecular weight protein, the *himA* gene product (Miller & Friedman, 1980; Miller & Nash, 1981). The site-specific recombination protein has a dual function in the *E.coli* cell: it is a subunit of the integration host factor (IHF) required for integrative recombination by lambda DNA (Miller & Nash, 1981)(the 20 500 molecular weight IHF protein consistes of two polypeptides); it also regulates the efficient expression of the *int* and *cI* genes specific for the lysogenic response of lambda (Miller, 1981). In addition, the *himA* gene product appears to participate in the normal growth of bacteriophage Mu and the precise excision

of transposable antibiotic resistance determinants (Miller & Friedman, 1980).

The *himA* gene product is induced to high levels by treatments such as UV irradiation and mitomycin C, and Miller *et al* (1981) showed that the *himA* gene is co-ordinately regulated by the *lexA* gene product and its own gene product. They suggest that the *himA* gene is part of the inducible SOS response because of its function as an "acquisitionase" for new genetic material through site-specific recombination. Such a function enhances the survival capability of a cell population under conditions of environmental stress.

We have previously reported the existence of the UV repair phenomena, LHR and phage reactivation, in the anaerobe *B.fragilis* (Jones & Woods, 1981; Slade *et al*, 1983b), but the presence, number and molecular weights of UV damage-inducible proteins in this anaerobe have never been reported. We investigated the induction of proteins by far-UV light in *B.fragilis* under anaerobic conditions, as well as the effect of the DNA repair protein inhibitors, caffeine and sodium arsenite on the induction of these UV proteins and on the survival of the irradiated *B.fragilis* cells under similar anaerobic conditions.

3.2 MATERIALS AND METHODS

3.2.1 Bacteria and media

The studies on the induction of proteins by far-UV irradiation were carried out on a *B.fragilis* Bf-2 strain which has been described in section 2.2.1. BHI broth and agar, supplemented with hemin, menadione and cysteine were used for bacterial propagation at 37°C. Prereduced one-quarter-strength Ringer solution was used as a dilution buffer (Jones & Woods, 1981). Irradiation and radioactive labelling of the cells were carried out in a defined minimal medium (Varel & Bryant, 1974). All experiments were performed under stringent anaerobic conditions in an anaerobic glove cabinet (Forma Scientific, Marietta, Ohio).

3.2.2 UV irradiation

Stationary phase overnight cultures of *B.fragilis* were obtained by subculturing a loopful of cells from a plate culture into BHI broth and incubating for 16 to 18 h at 37°C. These overnight cultures were diluted 100-fold into minimal medium and reincubated until the cultures reached a turbidity of 0,2 at 600nm ($1x10^8$ to $2x10^8$ c.f.u. ml^{-1}.)

Samples (9 ml) of these cultures were irradiated in open glass petri dishes with a Fluotest Piccolo Hanau Quartz germicidal lamp which emitted the majority of its output at 254 nm. The cell suspensions were continuously agitated during irradiation. The dose rate was measured with a Blak-Ray UV meter (model J-225; UV Products Inc., San Gabriel, Calif.) and samples were irradiated at a dose rate of 1,0 J $m^{-2}s^{-1}$. Survival curves of cells irradiated with increasing fluences were determined and labelling experiments were routinely carried out with cultures irradiated to a survival level of $\sim$ 0,1%.

3.2.3 Postirradiation treatment and labelling

Cell samples (2 ml) were removed from the irradiated culture and pulse-labelled for 10 min at 37°C by the addition of [^{35}S] methionine (40 µCi ml^{-1}) at different time intervals after UV irradiation. The labelled methionine was supplied by The Radio-chemical Centre, Amersham, England, and the final concentration of methionine used was 10 µg ml^{-1}. The cells were collected by centrifugation with a microfuge, washed twice with the Ringer solution and resuspended at a 20-fold concentration of the original sample volume in electrophoresis buffer

containing sodium dodecyl sulphate (SDS). The samples were then boiled for 2 min and either resolved immediately by polyacrylamide gel electrophoresis (PAGE) or stored at -20°C until required.

3.2.4 Effect of caffeine and sodium arsenite

The effect of the DNA repair protein inhibitors, caffeine and sodium arsenite, on the production of UV-induced proteins and on the survival of *B. fragilis* cells before and after irradiation were determined.The minimal inhibitory concentrations for caffeine and sodium arsenite were 2,5 mg ml^{-1} and 130 µg ml^{-1}, respectively. Caffeine (1 mg ml^{-1}) or sodium arsenite (100 µg ml^{-1}) was added to the broth cultures immediately after UV irradiation. In order to study the effect of the inhibitors on the survival of the cells, the cultures were either plated directly onto BHI agar plates containing the inhibitors or were treated with the inhibitors for specific time intervals before the inhibitors were diluted out and the surviving cells plated onto BHI agar plates in the absence of the inhibitors.

3.2.5 Electrophoresis and autoradiography

The labelled proteins were subjected to discontinuous SDS - 8,4% PAGE according to the methods described by Laemmli (1970) and O'Farrell (1975). Some of the basic solutions described by O'Farrell were slightly modified and these modifications are described in Media and Solutions (Appendix I).

The samples (20 µl per lane) were stacked at 100 V and then resolved either on 0,5 x 140 x 170 mm slab gels at 80V for 7 h at room temperature, or on 0,75 mm-thick Hoefer slab gels at 30mA per gel for 2,5 h at 4°C. The gels were stained with Coomassie brilliant blue (0,05%, wt/vol), destained, washed three times with distilled water and then dried onto Whatman No.1 filter paper under heat-vacuum. In order to increase the elasticity of the gels and so minimize mechanical damage to the gels during the drying process, 1% glycerol was added to the final wash. Some of the dried gels were treated with En^{3}Hance (New England Nuclear) according to the manifacturer's specifications before the gels were exposed to X-ray film. Labelled protein bands were visualized by exposing the dried gels to Kodak X-Omat MA X-ray film at -20 C for 21 to 28 days (Lasky & Mills, 1975).

The molecular weights of the newly induced UV proteins were calculated by comparing their electrophoretic mobility with that of protein standards of accurately known molecular weights. Molecular weight marker proteins were supplied by BDH Biochemicals Ltd. (Poole, England) and consisted of cross-linked polymers of a purified degradation product of myoglobin with a molecular weight range from 14 300 to 71 500. Purified human transferrin (molecular weight of 90 000; Sigma Chemical Co., St Louis, Mo.) was used as an additional marker. The autoradiograph strips were scanned with a Beckman Du-8 spectrophotometer with a gel scanner attachment.

3.3 RESULTS

3.3.1 UV induction of proteins

B.fragilis cells irradiated in minimal medium at a fluence of 75 J m^{-2} showed a level of survival of 0,1%. Under these conditions, filament formation or clumping did not occur and did not cause a sampling problem in the labelling experiments. The incorporation of [^{35}S] methionine into irradiated cells (0,1% survival) was linear over 10 min.

The induction of a new protein of molecular weight

95 000 (protein 1) and the induced synthesis of two other proteins with molecular weights of 90 000 and 70 000 (proteins 2 and 3, respectively) were observed after UV irradiation of *B.fragilis* cells under anaerobic conditions in 11 independent experiments (Fig 3.1 and 3.2). The 90 000- and 70 000-molecular weight protein (proteins 2 and 3, respectively) were synthesized in small amounts in unirradiated cells. Densitometric comparison of [^{35}S] methionine-labelled protein bands after SDS-PAGE of extracts from irradiated and unirradiated cells indicated that except for protein 1, 2 and 3, the proteins showed relatively minor variations after UV irradiation (Fig 3.2). The induction of a protein with a molecular weight between 37 000 and 40 000 was not observed in any of the 11 different UV irradiation and autoradiograph experiments.

3.3.2 Kinetics of induction

There was a rapid increase in the amount of proteins 1, 2 and 3 (molecular weights of 95 000, 90 000 and 70 000, respectively) over a 35-min period after UV irradiation (Fig 3.1). After the maximum level of induction was reached, as judged by the intensity of the autoradiograph bands, the proteins decayed with a half-life of about 20 to 25 min (Fig 3.3).

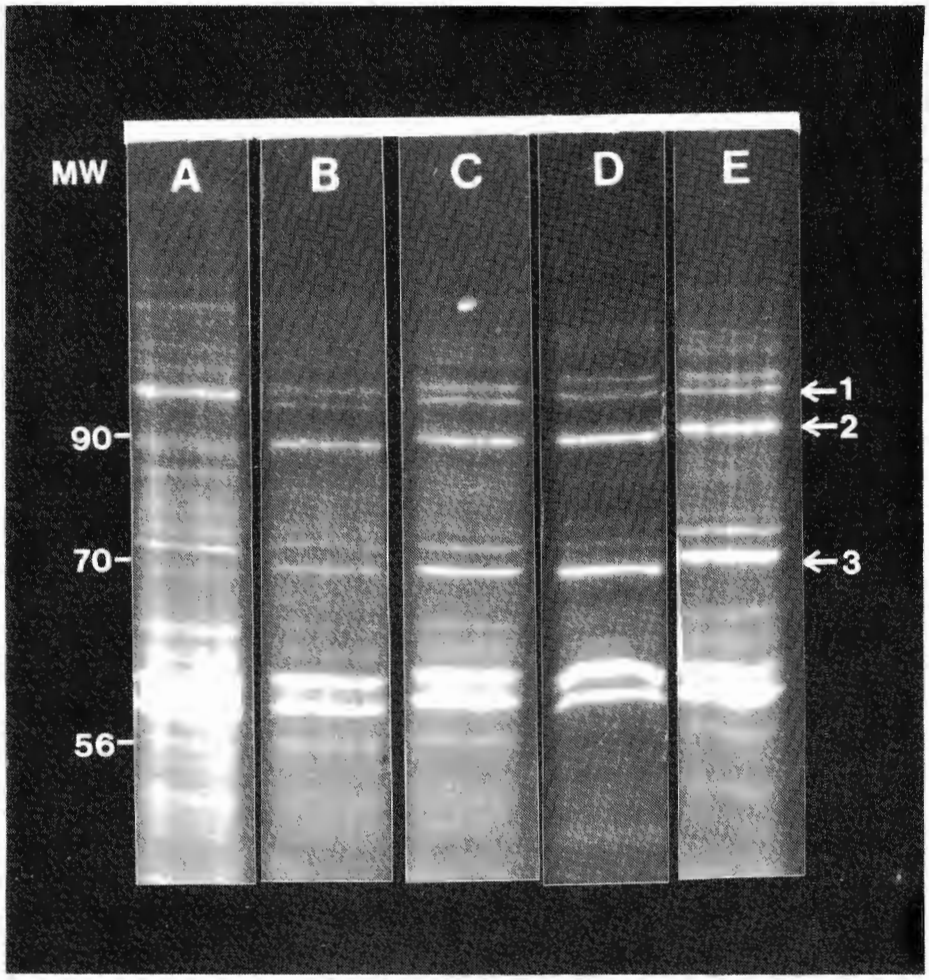

FIG 3.1 SDS-PAGE of extracts of far-UV-irradiated *B.fragilis* cells. The lanes represent: unirradiated cells (A) and irradiated cells labelled immediately (B), 15 min (C), 25 min (D), and 35 min (E) after irradiation. MW, Molecular weight (10^3). The arrows (1, 2 and 3) indicate the positions of the UV light-induced proteins.

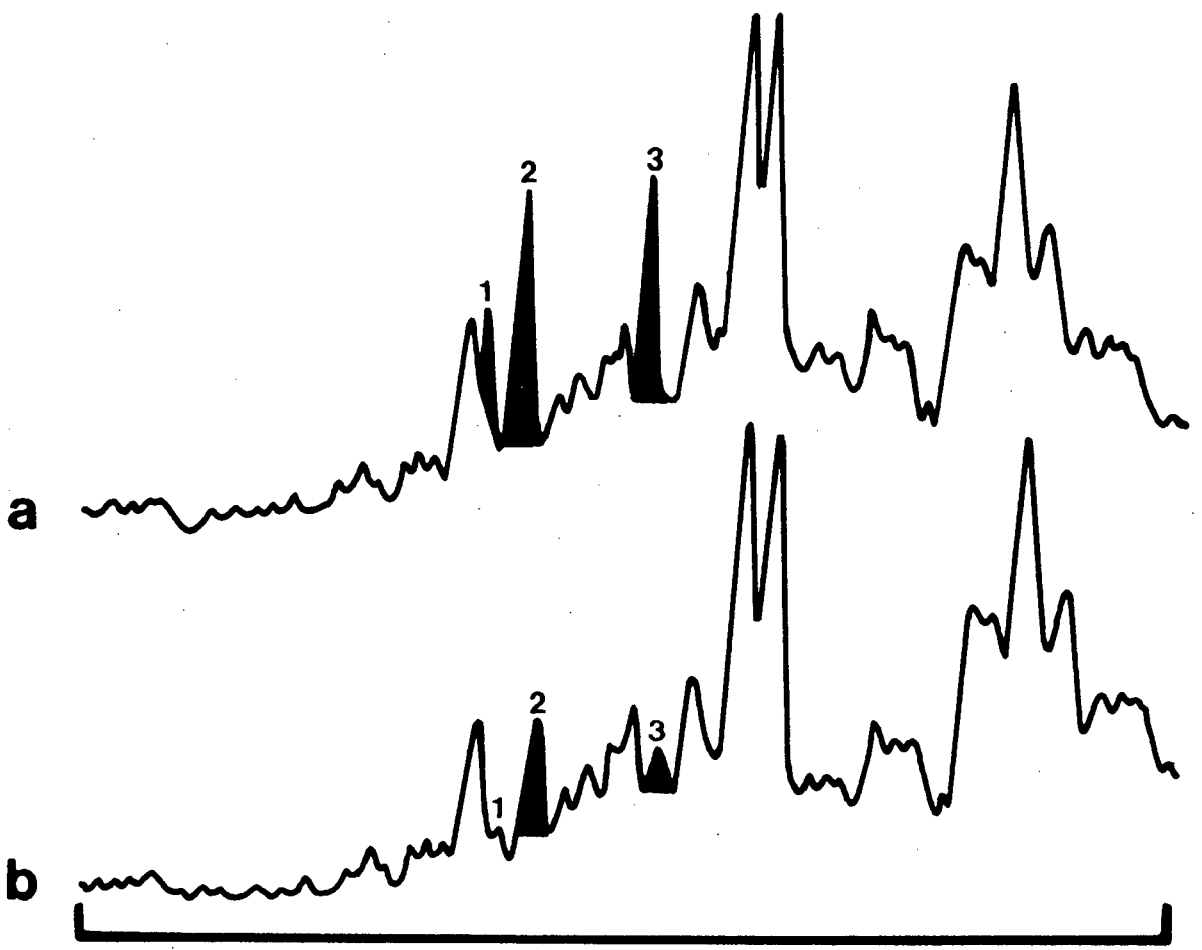

FIG 3.2 Densitometric comparison of [^{35}S] methionine-labelled protein bands after SDS-PAGE of extracts of far-UV-irradiated (a) and unirradiated (b) *B.fragilis* cells. Cells were irradiated to a survival level of 0,1% and labelled for 10 min at 15 min after UV irradiation. The UV light-induced bands of molecular weight 95 000, 90 000 and 70 000 are indicated by 1, 2 and 3, respectively.

The UV-induced protein 1 (molecular weight 95 000) was no longer detectable after 100 min of broth incubation following UV irradiation, while the levels of proteins 2 and 3 (molecular weights 90 000 and 70 000, respectively) at this time interval were similar to that in uninduced cells (Fig 3.3). The rate of synthesis of these induced proteins was dependent on the total UV fluence, but was independent of the dose rate at which the cells were irradiated (unpublished results).

3.3.3 Effect of caffeine and sodium arsenite

We previously observed that caffeine inhibited LHR (Jones & Woods, 1981) and phage reactivation (Slade *et al*, 1983a and 1983b; J.R.Parker, personal communication). Sublethal concentrations of caffeine caused a marked decrease in the number of colony-forming units after UV irradiation under anaerobic conditions (Fig 3.4). Similar results were obtained by Jones *et al* (1980). In these experiments the irradiated *B.fragilis* cells were plated onto caffeine-containing (1 mg ml^{-1}) agar plates immediately after UV irradiation. The most pronounced effect of caffeine involved the reduction in size of the shoulder of anaerobic UV survival curves (Fig 3.4). Time course viability studies

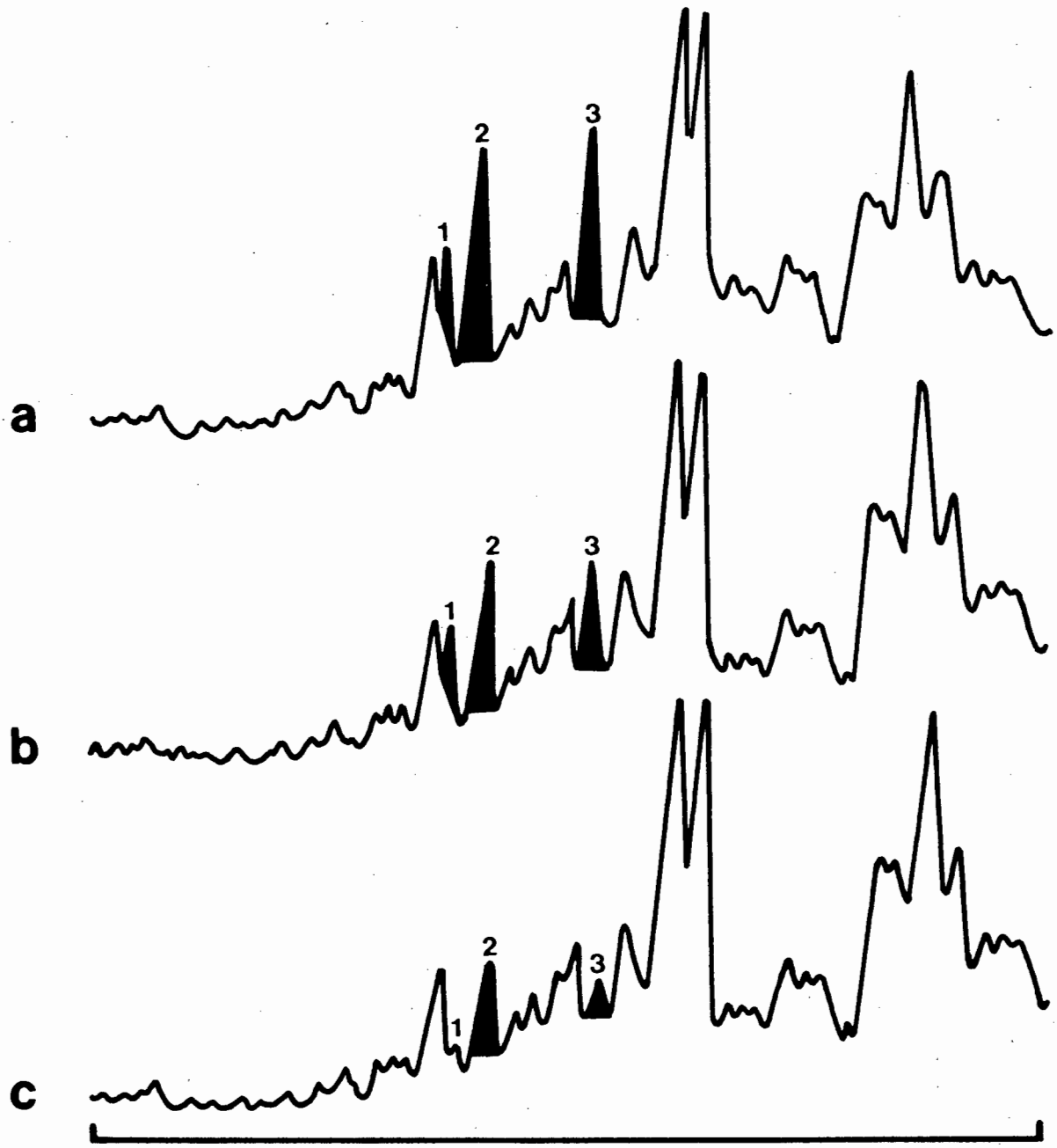

FIG 3.3 Densitometric comparison of [^{35}S] methionine-labelled protein bands after SDS-PAGE of extracts of far-UV irradiated *B.fragilis* cells. Cells irradiated to a survival level of 0,1% and labelled for 10 min at 35 (a), 60 (b), and 100 min (c) after UV irradiation. The UV light-induced proteins of molecular weight 95 000, 90 000 and 70 000 are indicated by 1, 2 and 3, respectively.

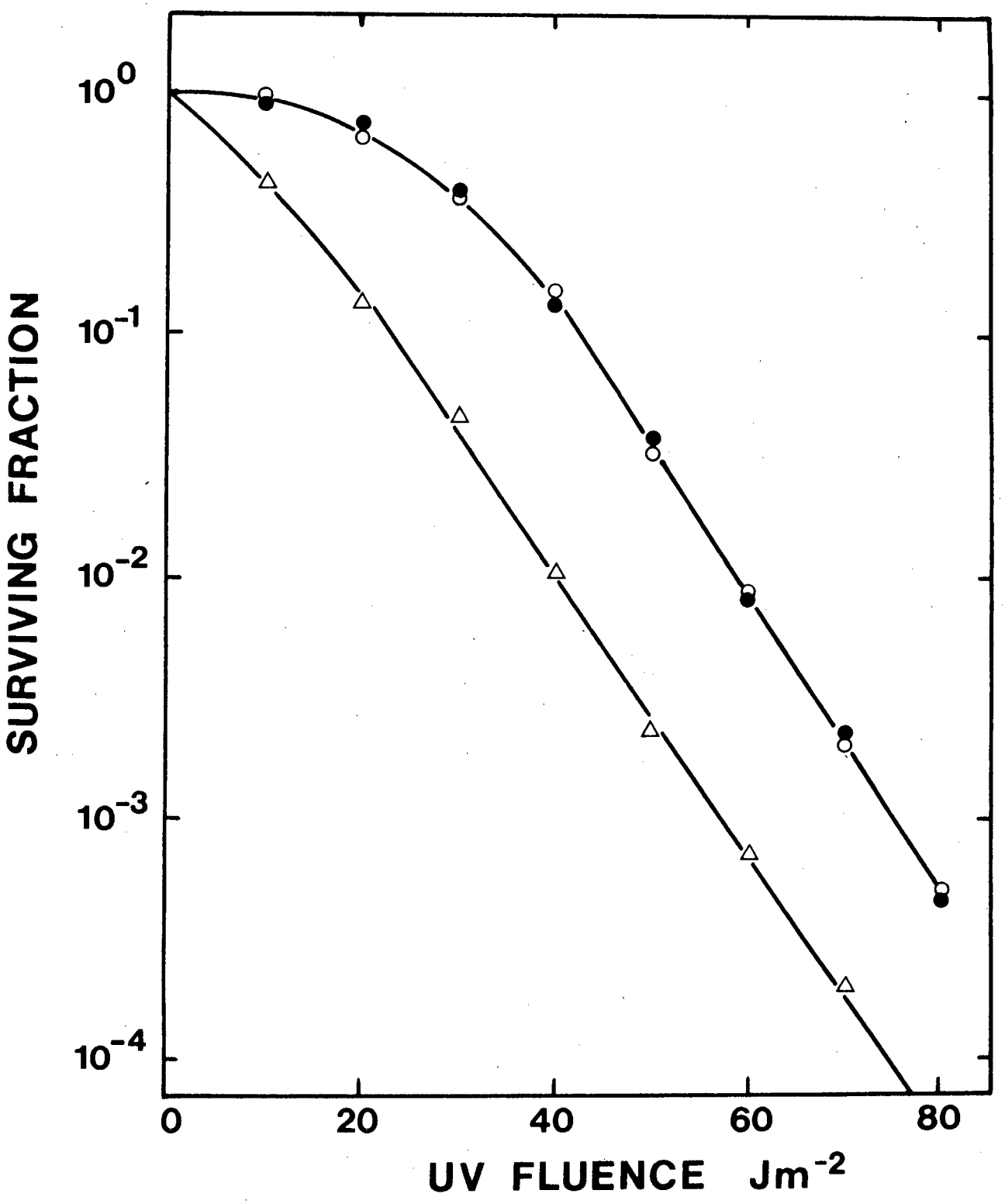

FIG 3.4 The effect of caffeine and sodium arsenite on the survival of *B.fragilis* cells irradiated with increasing UV fluences. Cells irradiated anaerobically and plated onto brain heart infusion agar plates with sodium arsenite (100 μg ml^{-1}) (O), caffeine (1 mg ml^{-1}) (△) and without any inhibitors (●).

on the effect of caffeine on irradiated cells in minimal medium showed a rapid inhibition of colony formation, followed by a slow decrease in viability (Fig 3.6). Unirradiated cells were relatively unaffected by the presence of 1 mg ml^{-1} caffeine. The addition of caffeine immediately after UV irradiation specifically inhibited the induction of the 95 000-, 90 000- and 70 000-molecular weight proteins (proteins 1, 2 and 3 respectively) (Fig 3.5). After treatment with caffeine, the 95 000-molecular weight protein was absent, and the concentrations of the 90 000- and 70 000-molecular weight proteins were similar to those in unirradiated cells. The addition of caffeine 15 min after UV irradiation had no effect on the induction of proteins 1, 2 and 3 (molecular weights 95 000, 90 000 and 70 000, respectively) (unpublished results).

When cells were plated onto sodium arsenite plates (100 $\mu g\ ml^{-1}$) after irradiation under anaerobic conditions, no decrease in the number of colony forming units occurred (Fig 3.4). Time course viability studies showed only a slight decrease in the rate of colony formation over 90 min after the addition of sublethal concentrations of sodium arsenite to the irradiated and unirradiated *B. fragilis*

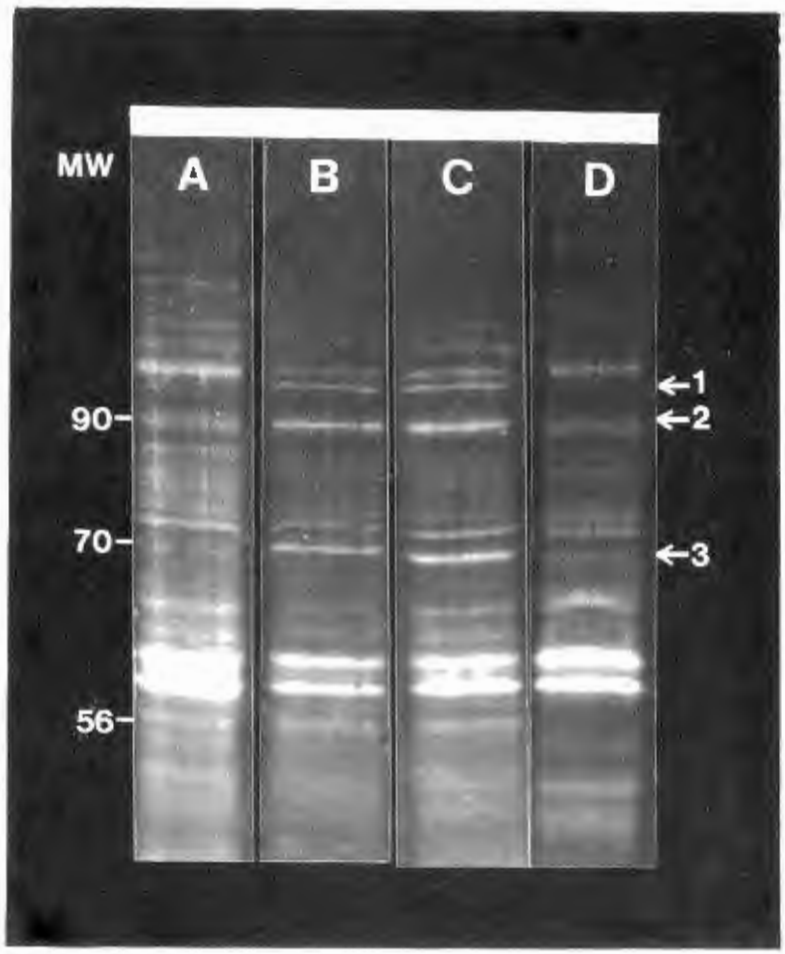

FIG 3.5 Effect of caffeine and sodium arsenite on the induction of proteins by far-UV light in *B.fragilis*. SDS-PAGE of extracts of unirradiated cells (lane A) and irradiated cells labelled 15 min after irradiation (lane B). Sodium arsenite (100 µg ml^{-1}) (lane C) and caffeine (1 mg ml^{-1}) (lane D) were added immediately after irradiation, and the cells were held for 15 min before labelling. MW, Molecular weight (10^3). The arrows (1, 2 and 3) indicate the positions of the UV light-induced proteins.

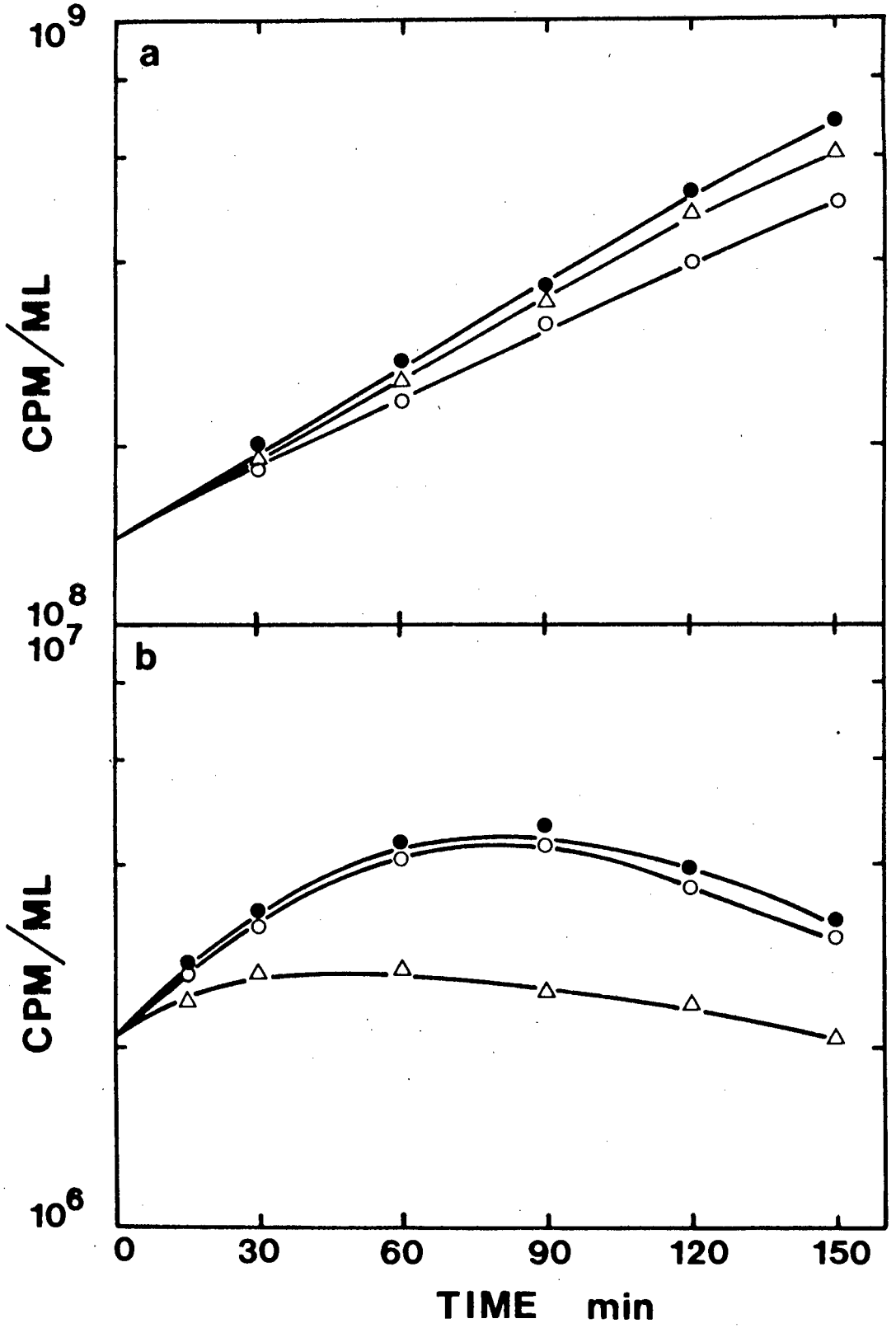

FIG 3.6 The effect of caffeine and sodium arsenite on the viability of unirradiated (a) and far-UV irradiated (b) *B.fragilis* cells. Unirradiated anaerobic cells incubated with caffeine (1 mg ml^{-1}) (△), sodium arsenite (100 µg ml^{-1}) (○) and without any inhibitors (●). (b) Cells irradiated to a survival level of 1,5% and incubated with caffeine (1 mg ml^{-1}) (△), sodium arsenite (100 µg ml^{-1}) (○) and without any inhibitors (●).

cultures (Fig 3.6). The addition of 100 $\mu g\ ml^{-1}$ of sodium arsenite immediately after UV irradiation did not affect the production of the three UV-induced proteins (Fig 3.5).

3.4 DISCUSSION

Exposure of *B. fragilis* cells to far-UV light resulted in the induction of one novel protein (molecular weight 95 000) and the increased synthesis of two proteins (molecular weights of 90 000 and 70 000) which were synthesized in small amounts in un-irradiated cells (Schumann *et al*, 1982). It is tempting to associate the greatly enhanced synthesis of these three proteins with the need for protein synthesis in the period subsequent to irradiation and thus to attribute to these proteins a role in the process of DNA repair. We have previously reported various far-UV-induced repair systems in the anaerobe *B. fragilis* (Jones & Woods, 1981; Slade *et al*, 1983b).

Direct comparison with other organisms can be quite complex since there is no assurance that a protein with similar function has the same molecular weight, nor that it is regulated in the same way. Comparison with the *E. coli* proteins may nevertheless be instruc-

tive in the case of the anaerobe *B.fragilis* for which no UV-sensitive or -resistant mutants have as yet been isolated.

The action of DNA repair protein inhibitors on the induction of the three proteins in *B.fragilis* is very interesting. The addition of sublethal concentrations of caffeine to irradiated *B.fragilis* cells under anaerobic conditions inhibited the induction of the three UV-inducible proteins; it also inhibited LHR (Jones & Woods, 1981) and phage reactivation (Slade *et al*, 1983a and 1983b) in this organism, and caused the reduced survival of UV-irradiated *B.fragilis* cells. On a molecular level caffeine inhibited UV-induced DNA degradation and the subsequent synthesis of DNA in irradiated *B. fragilis* cells (Chapter 2). In *E.coli* the uvr^+-dependent functions of excision repair and HCR are inhibited by caffeine (Rupert & Harm, 1966; Setlow, 1967; Fong & Bockrath, 1979; Rothman, 1980). Two of the *E.coli* uvr^+ proteins, $uvrA^+$ (Kenyon & Walker, 1981) and $uvrB^+$ (Fogliano & Schendel, 1981), were recently shown to be inducible. UV survival and the induction of the three UV-inducible proteins were not affected by sodium arsenite in *B.fragilis*; sodium arsenite is thought to inhibit $recA^+$-dependent repair processes in *E.coli*. It is therefore possible

that the three inducible proteins may be involved in excision repair processes in *B.fragilis*.

The anaerobe *B.fragilis* differs from *E.coli* in that a protein of molecular weight between 37 000 and 40 000, corresponding to the *E.coli recA*$^+$ protein is not observed after induction by far-UV light. The absence of such an inducible protein does not, however, exclude the possibility that a protein with a similar *recA*$^+$-type function has a higher molecular weight in *B.fragilis* or that a *recA*-type protein is produced constitutively in *B.fragilis* at low basal levels undetectable by PAGE. The low basal levels of *recA*$^+$ protein constitutively present in uninduced *E.coli* cells are sufficient for *recBC*-dependent general recombination. A constitutively active recombinational pathway is also responsible for 70% of the postreplicative gap-filling in irradiated *Micrococcus luteus* cells (Tomilin & Zherebtsov, 1982; Zherebtsov & Tomilin, 1982). There is some doubt about the inducibility of a *recA*$^+$-type protein in *Haemophilus influenzae* as well, since Kimball *et al* (1977) failed to detect enhanced postreplication repair in *H.influenzae* cells subjected to low UV doses.

Devoret (1981) showed that Weigle reactivation in

E.coli does not require the induced synthesis of the *recA*$^+$ protein, and the results of Thompson & Hart (1981) on Weigle reactivation and UV mutagenesis in *Staphylococcus aureus* were also thought to be compatible with a hypothesis of DNA repair in which *recA*$^+$ protein is constitutively present in the cells. Inducible excision repair, however, appears to be an important component of the Weigle reactivation system in *E.coli* (Cooper, 1981). Weigle-type reactivation of UV-irradiated phage has recently been demonstrated in *B.fragilis* cells (Slade *et al*, 1983b) and this repair phenomenon was inhibited by the addition of sublethal concentrations of caffeine (J.R. Parker, personal communication).

The low constitutive level of *recA*$^+$ protein in *E.coli* cells is also sufficient to derepress SOS operons, given the appropriate inducing treatment to activate its protease activity (Bailone *et al*, 1979; Devoret, 1981). In *E. coli* DNA degradation products generated after UV irradiation of the cells, serve as effective inducing signals for the initiation of SOS events (Oishi *et al*, 1978 and 1981). Anaerobic UV irradiation of *B.fragilis* cells caused extensive DNA degradation (Chapter 2) and the time and

extent of DNA degradation correlated well with the maximum induction of the three UV proteins.

There is therefore no firm evidence against the possible existence of a *recA*-type protein in *B. fragilis* but it is, however, very interesting that the mapping of the *B.fragilis* chromosome by chromosome mobilization has been unsuccessful and confirmation of a true prophage relationship in this organism is also lacking. Furthermore, the only reports to date of chromosomal recombination in *B.fragilis* involve antibiotic resistant elements which resemble transposition elements, and site-specific recombination (Mays *et al*, 1982). Site-specific recombination in *E.coli* is facilitated by the damage-inducible *himA* protein (Miller *et al*, 1981), which is also responsible for the precise excision of transposable antibiotic resistance determinants (Miller & Friedman, 1980). The resolution limitations of an 8,4% - SDS polyacrylamide gel system prevented the observation in *B.fragilis* cells of an 11 000-molecular weight protein similar to the *himA* protein of *E.coli* (Miller & Nash, 1981).

These 8,4%-SDS polyacrylamide gels also did not

allow the detection of an 18 000-molecular weight protein similar to the *E.coli sfiA*$^+$ protein. Although no detailed characterization of UV-induced filamentation in *B.fragilis* has yet been carried out, filamentation in this organism differs from that in *E.coli* in that it is fluence-dependent and *B.fragilis* filaments always contain a certain number of septa (Jones, 1979; Jones & Woods, 1981). The *sifA*$^+$-dependent regulation of cell division in *E.coli* is UV fluence-independent and the induction of this protein is triggered by even minor perturbations of DNA replication (Quillardet *et al*, 1982).

Induced mutagenesis (error-prone repair) in *E.coli* is not only due to a functional *recA* protein (Hanawalt *et al*, 1981) but also appears to require the SOS-induced production of the 45 000-molecular weight *umuC*$^+$protein (Bagg *et al*,1981; Perry & Walker, 1982), which is postulated to inhibit the error-correcting 3' → 5' exonucleolytic activity of bacterial polymerases (Villani *et al*, 1978; Devoret, 1981). A 45 000-molecular weight protein was not induced by UV irradiation in *B.fragilis* cells under anaerobic conditions, and *B.fragilis* is notoriously difficult to mutate by far-UV irradiation. The inability to sustain UV-induced mutations in *Micrococcus radiodurans* (Sweet & Mosely, 1974 and 1976), in *H.influenzae* Rd (Kimball

et al, 1977) and in *Proteus mirabilis* (Hutchinson & Medill, 1954; Böhme, 1964) has been linked to the lack of error-prone repair system in these organisms. The failure to isolate UV mutants in spite of an intensive screening programme, suggests that *B.fragilis* may also lack an error-prone repair system.

The kinetics of induction and half-lives of the three UV-proteins in *B.fragilis* cells correlate well with that of the SOS functions induced in *E.coli*. The production of the UV-induced proteins in *B.fragilis* increased over a 35-min period, whereas the average time required for SOS functions in *E.coli* to reach a maximum level of induction is approximately 30 min (Witkin, 1975). UV mutagenesis and Weigle reactivation in *E.coli* decay with a half-life of 30 min once the optimum level is achieved (Witkin, 1976); the half-lives of the induced *B.fragilis* proteins were about 20 min and the levels of these proteins were similar to that in uninduced cells after 100 min under non-inducing conditions.

Although it could be speculated that the three UV-inducible proteins in *B.fragilis* are associated with the occurrence of far-UV-irradiated repair phenomena observed in this organism, studies with suitable *B.fragilis* mutants altered in their repair

capabilities are essential to clarify the functions of the inducible proteins.

CHAPTER 4

THE EFFECT OF OXYGEN AND HYDROGEN PEROXIDE ON NUCLEIC ACID AND PROTEIN SYNTHESIS IN UNIRRADIATED AND UV-IRRADIATED *BACTEROIDES FRAGILIS* CELLS

4.1 INTRODUCTION

Oxygen is toxic to all living organisms (Haugaard, 1968; Gottlieb, 1971), but microorganisms differ in their sensitivity to oxygen and a continuous spectrum of oxygen tolerance, from the most strict anaerobe to the least sensitive hyperaerobe, can be discerned (Morris, 1976). The obligate anaerobes as a group have drawn the attention because of their adverse sensitivity to molecular oxygen, but despite extensive research, the physiological and biochemical nature of oxygen toxicity in anaerobes remains poorly understood. There are two main hypotheses which suggest that either molecular oxygen itself is the toxic agent by virtue of its excellence as an oxidant, or that oxygen exerts its toxic effects via oxygen free radicals produced during the interaction of oxygen with organisms and/or components in their culture media. Comprehensive reviews on the physiology of obligate anaerobes and their relationship with oxygen have been published by Morris (1975 and 1976).

Irreparable damage to DNA is thought to be one of the main causes of loss of viability in microorganisms. *B.fragilis* has been described as a moderate anaerobe because it can withstand exposure to air for at least 60 min without loss of viability (Loesche, 1969). Stevenson (1979) and Glass *et al* (1979) reported that macromolecular synthesis in *Bacteroides thetaiotaomicron* and *Bacteroides ruminicola* is inhibited immediately after exposure to oxygen. No studies on the kinetics of macromolecular synthesis in *B.fragilis* cells in the presence of oxygen have been reported.

An interesting finding is that oxygen sensitizes *B.fragilis* cells to the far-UV irradiation (Jones *et al*, 1980). In all other bacteria studied so far, inactivation by far-UV light is independent of the presence of oxygen (Zetterberg, 1964; Webb & Lorenz, 1970; Peak *et al*, 1973; Webb, 1977; Peak *et al*, 1981). However, it was found that oxygen sensitizes bacterial cells to killing by ionizing radiation by modifying the chemical nature of the radiation-induced DNA lesions and so affecting the amount of repair that can be brought about by the various enzyme repair systems (Johansen *et al*, 1974; Koch & Painter, 1975; Tallentire, 1981). In *B.fragilis* oxygen does not affect the number of pyrimidine dimers

produced by UV radiation (Jones & Woods, 1981) and Slade *et al* (1981) found that the cells are more sensitive to UV radiation in the presence of oxygen rather than to oxygen after irradiation. The effect of both oxygen and UV irradiation on macromolecular synthesis in *B.fragilis* (or any other anaerobe) has not been reported as yet.

4.1.1. The chemistry and biochemistry of oxygen and oxygen radicals

Molecular oxygen usually exists in its kinetically inert ground state which is called triplet oxygen. The triplet oxygen has limited biological activities (Fridovich, 1978; McCord, 1979; Fee, 1981) and is also relatively insoluble in aqueous media (Brown, 1970). Oxygen, however, possesses the potential of being a powerful oxidising agent in biological redox reactions. It has two outer orbitals with only one unpaired electron each and can thus accommodate an additional electron in each orbital. Complete reduction of oxygen to water requires four electrons and preferentially occurs via an univalent reduction process (Badwey & Karnovsky, 1980).

4.1.1.1 Superoxide

The one-electron reduction of molecular oxygen

produces two very damaging oxygen radicals, the superoxide radical in its ionized form and the hydroxyperoxy radical in its protonated form. The more powerful oxidizing form, the hydroxyperoxy radical, is only present in very small amounts at physiological pH values around 7,4 (Halliwell, 1982). Fluxes of superoxide anions are generated by various photochemical and enzymatical methods. Reduced flavins, flavoproteins, quinones, thiols, iron sulphur proteins and tetrahydropteridine can all reduce molecular oxygen to superoxide (Morris, 1976). Superoxide is also a transient product of normal aerobic respiration (Fridovich, 1975). Phagocytic leukocytes and activated macrophages produce superoxide anions during greatly enhanced respiratory bursts in order to destroy ingested particles and to achieve the nonphagocytic lysis of tumour cells (Badwey & Karnovsky, 1980; Wilson *et al*, 1980). Both the para-quinone streptonigrin (Fridovich, 1974) and the redox dye paraquat (I,I'-dimethyl-4,4'-bipyridinium-dichloride) (Farrington *et al*, 1973; Hassan & Fridovich, 1978 and 1979) cause a decrease in viability of bacteria in aerobic media by their ability to increase the intracellular flux of superoxide radicals (Moody & Hassan, 1982). The pronounced oxygen effect observed for the action of mitomycin C in

S. typhimurium is also due to its ability to generate superoxide radicals (Moody & Hassan, 1982).

Superoxide anions may be productively utilized by aerobic bacteria in controlled hydroxylation reactions, but the uncontrolled generation and accumulation of superoxide radicals leads to serious damage of vital cell components (Morris, 1976). Superoxide radicals are more soluble in organic solvents than in water and tend to be concentrated in the lipophilic interior of cell membranes. There they can deacylate phospholipids by a nucleophilic attack upon ester bonds, and oxidize tocopherols, so destroying one of the membrane's defences against lipid peroxidation (Halliwell, 1982). The induced peroxidation of membrane lipids leads to a loss of membrane potential, increased leakiness and decreased membrane fluidity. Superoxide radicals are also capable of oxidizing bile pigment and enzyme-bound NADH (Bielski & Chan, 1976; Fridovich, 1981; Halliwell, 1982). They can destroy tryptophan residues in proteins (Green & Crusov, 1968), depolymerize acid polysaccharides and oxidize labile -SH groups in the cell (Halliwell, 1982). A biologically important damaging action of superoxide radicals is that they can cause single-strand breaks in DNA (Moody & Hassan, 1982) either directly or by the secondary production of other radicals that attack DNA.

Superoxide radicals can be removed from the system by the pH-dependent spontaneous dismutation of two superoxide radicals to form hydrogen peroxide and molecular oxygen (Badwey & Karnovsky, 1980). Superoxide radicals cannot accept electrons directly because of the instability of the peroxy ion, but they can effectively oxidize metal ions and thus react as oxidizing agents via metal-superoxo complexes.

4.1.1.2 Hydrogen peroxide

Hydrogen peroxide is one of the most stable oxygen radicals and can accumulate appreciably in neutral aqueous media. It is produced in most aerobically grown cells (Lemberg & Legge, 1949) through the two-electron reduction of oxygen, generally mediated by reduced flavoproteins (Malmström, 1982).

Hydrogen peroxide is almost ubiquitous in autoclaved culture media exposed to air. The heating of glucose and phosphate together in culture media produces intermediates which generate hydrogen peroxide upon aeration of the media (Carlsson *et al*, 1978). The oxidation of the thiol group of the cysteine present in anaerobic media also produces hydrogen peroxide when the culture media are exposed to air (Carlsson *et al*, 1979).

An increased yield of single-strand DNA breaks and enhanced lethality were observed in *E.coli* (Pollard & Weller, 1967; Ananthaswamy & Eisenstark, 1977; Hartman & Eisenstark, 1978; Carlsson & Carpenter, 1980), *S.typhimurium* (Yoakum & Eisenstark, 1972; Carlsson & Carpenter, 1980), and T7 phage (Ananthaswamy & Eisenstark, 1976), after treatment with hydrogen peroxide. Hydrogen peroxide affects isolated DNA by altering the DNA so that all four bases are liberated (Uchida *et al*, 1965; Yamafugi & Uchida, 1966; Freese *et al*, 1967; Rhaese & Freese, 1968; Massie *et al*, 1972). All studies on isolated DNA, however, were carried out using relatively high concentrations of hydrogen peroxide (0,05 to 0,1 M) and long periods of incubation in the presence of ferrichloride. In heat-treated bacteria hydrogen peroxide appears to interfere with the ability of the cells to recover after stress (Martin *et al*, 1976; Brewer *et al*, 1977; Flowers *et al*, 1977; Rayman *et al*, 1978). It also displays a synergistic effect with near-UV and Hartman & Eisenstark (1978) proposed that hydrogen peroxide decreases *recA*$^+$ -dependent repair of the induced DNA damage. The latter synergism was thought to be specific for near-UV (Hartman & Eisenstark, 1978), but Bayliss & Waites (1979) found that the simultaneous treatment of dormant spores of *B.fragilis* with far-UV radiation

and hydrogen peroxide resulted in a 2 000-fold greater kill than that produced by irradiation alone or followed by a treatment with hydrogen peroxide. This synergism was not due to the hydroxyl radicals formed during the decomposition of hydrogen peroxide by UV irradiation, since hydroxyl radical quenchers failed to protect the spores.

Additional damage attributable to hydrogen peroxide includes the inhibition of key enzymes involved in energy metabolism and a repairable injury affecting cell division in *Serratia marcescens* (Campbell & Dimmick, 1966), paralysis of the respiratory chain in *E.coli* (Frey & Pollard, 1968) and some hydrogen peroxide-induced DNA degradation in *E.coli* (Keller & Pollard, 1977).

4.1.1.3 Hydroxyl radicals

The hydroxyl radical is the most reactive of the various oxygen radicals and this very potent oxidizing agent can attack all of the organic substances in the cell. Hydroxyl radicals are capable of indiscriminately reacting with and damaging nucleic acids (Cadet & Teoule, 1978; Ito, 1978; Lynch & Fridovich, 1978; Totter, 1981). They abstract hydrogen atoms from saturated carbon atoms of both purines and

pyrimidines at a rate constant close to the limit set by diffusion. Hydroxyl radicals can initiate lipid peroxidation of membranes by hydrogen atom abstraction from unsaturated lipids (Badwey & Karnovsky, 1980) and can also augment the yield of singlet oxygen by reacting with superoxide anions.

The hydroxyl radical is formed through the action of ionizing radiation on cells (Blok & Verhey, 1968; Achey & Duryea, 1974; Ewing, 1981), the decomposition of hydrogen peroxide by UV radiation, and by the ferric ion-catalyzed reaction between superoxide and hydrogen peroxide (Haber & Weiss reaction, proposed in 1934; Beauchamp & Fridovich, 1970). This last reaction was found to be a commonplace event in any aerated system where significant amounts of hydrogen peroxide accumulate (Morris, 1975) and many supporters of the superoxide theory have settled on this reaction as the molecular basis of oxygen toxicity (Fee, 1982).

4.1.1.4 Singlet oxygen

Singlet oxygen represents an electronically excited state of triplet oxygen in which the two outer electrons achieve antiparallel spin, either in the same orbital or in separate orbitals (Morris, 1975 and 1976). It is produced by a variety of chemical

means, as well as through the action of certain enzyme systems.

Singlet oxygen can react in a strong electrophilic fashion with molecules like DNA which possess high electron densities (Cadet & Teoule, 1978; Lynch & Fridovich, 1978). It is proposed to add dienes and unsaturated ring compounds to purines and pyrimidines (Kearns & Khan, 1969) and the susceptibility of the four DNA nucleotides to oxidation by singlet oxygen decreased in the order dG>>dT>dC≃dA. Although singlet oxygen is rapidly quenched by water (Hewitt & Morris, 1975; Morris, 1976), it would not be solvated so easily in the hydrophobic lipid/protein cell membranes and could react directly with poly-unsaturated fatty acids to form hydroperoxide (Halliwell, 1982).

4.1.2 Survival of anaerobes in oxygen

Although anaerobes differ markedly in their sensitivity to oxygen (Fredette *et al*, 1967; Loesche, 1969; Tally *et al*, 1975; Walden & Hentges, 1975; Rolfe *et al* 1977 and 1978), the presence of molecular oxygen inhibits growth of all obligate anaerobes (Loesche, 1969; O'Brien & Morris, 1971; Walden & Hentges, 1975, Hoshino *et al*, 1978; Rolfe *et al*, 1978, Wimpenny &

& Samah, 1978; Samah & Wimpenny, 1982) and prolonged exposure to oxygen invariably leads to cell death. Obligate anaerobes were divided into two groups by Loesche (1969): strict anaerobes which are unable to grow on plates when there is more than 0,5% oxygen in the atmosphere, and moderate anaerobes which can grow on plates in the presence of 2 to 5% oxygen. Certain factors may, however, affect the classification of anaerobes into the different groups: several strict anaerobes may become more aerotolerant after two to three subcultures (Willis, 1969) and cells in exponential phase in batch growth are usually more oxygen-sensitive than cells in stationary phase (Morris, 1976; Jones *et al*, 1980).

The physiological basis of the growth inhibitory and often lethal effect of oxygen is not fully understood. The response of anaerobes to oxygen can be divided into two phases: a reversible bacteriostatic phase during which growth of anaerobes is inhibited, and a second bactericidal phase during which the primary defences on the cell are overwhelmed and cell death ensues (Morris, 1975).

4.1.2.1 Bacteriostatic phase

Although anaerobes are unable to grow in oxygen,

several anaerobes can withstand considerable exposure to oxygen without significant losses in viability and these organisms can recover totally and rapidly if anaerobic conditions are restored without undue delay (Loesche, 1969; O'Brien & Morris, 1971; Onderdonk *et al*, 1976). O'Brien & Morris (1971) found that net DNA, RNA and protein synthesis are inhibited concomitantly with growth inhibition in aerated *Clostridium acetobutylicum* cells. Growth and the synthesis of the macromolecules resume at their normal rates when *C.acetobutylicum* cells are restored to anaerobic conditions.

Several primary defence mechanisms are involved in the protection of anaerobes against oxygen damage during this first bacteriostatic phase:

4.1.2.1.1 Radical scavenging enzymes

The oxygen free radical scavenging enzymes, catalase and superoxide dismutase, are present or are induced in many anaerobes on exposure to air (Holdeman & Moore, 1972; Hewitt & Morris, 1975; Carlsson *et al*, 1977; Ashley & Shoesmith, 1977; Tally *et al*, 1977; Gregory *et al*, 1978). These enzymes remove hydrogen peroxide, superoxide radicals and singlet oxygen from the aerated culture media before these radicals can

attack and damage cellular constituents. The anaerobe *Peptostreptococcus anaerobius* lacks both catalase and superoxide dismutase, but a NADH oxidase enzyme which reduces oxygen to water is induced in this organism in the presence of oxygen (Hoshino *et al*, 1978). *Selenomonas ruminantium* (Samah & Wimpenny, 1982) and *C.acetobutylicum* (O'Brien & Morris, 1971) also possess oxygen-inducible NADH oxidases which markedly enhance oxygen reduction upon exposure of these organisms to oxygen. *Lactobacillus plantarum* does not possess any of the defensive enzymes, but is capable of accumulating vast amounts of Mn^{2+} which can successfully scavenge superoxide radicals (Archibald & Fridovich, 1981).

4.1.2.1.2 Intracellular sulfhydryl groups

Sulfhydryl groups are suitable hydrogen donors which can readily give up a hydrogen atom to repair a DNA lesion caused by hydrogen abstraction by oxygen itself or by the hydroxyl radical (Howard-Flanders, 1960). The glutathione (GSH) synthetic pathway in *E.coli* (Michael *et al*, 1981) and mammalian cells (Nathan, 1982) has consequently been found to be closely linked to the prevention of oxidative injury in these cells. The oxidation of labile thiol groups, such as those present in low-potential carriers like

ferredoxin (Wimpenny & Samah, 1978), will detoxify oxygen at the expense of the inactivation of the thiol compound.

4.1.2.1.3 Reducing power

Oxygen is a much more avid electron acceptor than the normal terminal oxidants of fermentation (O'Brien & Morris, 1971). The primary electron donor NAD(P)H which is required for anaerobic biosynthesis, will thus preferentially react with oxygen, if present. This leads to the indirect inhibition of the energy-producing biosynthetic pathway and causes a concomitant growth inhibition and a rapid drop in intracellular ATP levels (O'Brien & Morris, 1971). The decrease in NADH concentration observed in *B.fragilis* and *C.acetobutylicum* cells under aerobic conditions also coincided with volatile fatty acid production in *B. fragilis* and butyrate production in *C.acetobutylicum* (O'Brien & Morris, 1971; Onderdonk *et al*, 1976).

Although an organism can normally divert a considerable part of its 'reducing power' to the energetically unrewarding task of detoxifying molecular oxygen (Morris, 1970; O'Brien & Morris, 1971), Morris (1976) proposed that anaerobes have a tightly coupled cycle of intracellular electron donation and acceptance and

would have little scope of diverting the electron flow for the purpose of reductive detoxification of oxygen. Certain aerobes (e.g. the nitrogen-fixing *Azotobacter* species) employ a similar mechanism of diverting reducing power to shield key oxygen-labile components in the cell from direct contact with oxygen (Drozd & Postgate, 1970; Jones *et al*, 1973).

4.1.2.1.4 Eh value and antioxidants

Several investigators claimed that a negative Eh value is a prerequisite for anaerobic growth (Hanke & Katz, 1943; Socransky *et al*, 1964; Fredette *et al*, 1967). Aeration increases the Eh value of cultures with a concomitant cessation of growth. The Eh value of a culture, however, is merely a measurement of the overall reduction-oxidation tendency of such a culture. O'Brien & Morris (1971), Walden & Hentges (1975) and Onderdonk *et al* (1976) all showed that growth of anaerobes could continue unchecked in cultures in which the Eh value was positively poised by the addition of potassium ferricyanide. *C.acetobutylicum*, *C.perfringens*, *B.fragilis* and *Peptococcus magnus* continued growing at normal anaerobic rates at Eh values of +300mV and higher, while the introduction of oxygen into these cultures immediately inhibited growth even when the cultures were poised

at -50mV by the addition of dithiothreitol (O'Brien & Morris, 1971; Walden & Hentges, 1975; Onderdonk *et al*, 1976).

Nevertheless, the low redox potential which is established in anaerobic culture media by the addition of antioxidants generally appears to improve the initiation of growth of anaerobes (Morris, 1975). These antioxidants can detoxify oxygen in the medium before it interacts with the cells and will ensure minimal drainage of reducing power from organisms, with the result that the reducing power can then be more productively utilized in energy-yielding biosynthetic pathways. Standard growth media for anaerobes therefore include supplements of at least one thiol compound such as hydrogen sulphide, cysteamine or cysteine plus or minus dithiothreitol, and sodium thioglycollate. Hydrogen, which is an essential part of the standard anaerobic gas mixture, can also effectively lower the culture Eh (Morris, 1976).

Oxidized reducing agents (such as cysteine), however, can be toxic for some fastidious anaerobes (Holdeman & Moore, 1975), *E.coli* (Kari *et al*, 1971; Brown, 1975), *Bacillus subtilis* (Villareja & Westley, 1966) and *Peptostreptococcus anaerobius* (Carlsson *et al*, 1979).

The damaging oxygen radical, hydrogen peroxide, is rapidly formed in the culture media during the metal ion-catalyzed autoxidation of cysteine in the presence of oxygen (Michaelis, 1929; Taylor *et al*, 1966); anaerobes can thus be protected against the toxic effect of aerated culture media by the addition of both catalase (Hoshino *et al*, 1978) and peroxidase (Carlsson *et al*, 1979).

4.1.2.2 Bactericidal phase

Excessive exposure of anaerobes to oxygen can overwhelm the primary defence mechanisms in these organisms. This will allow the free entry of oxygen and oxygen free radicals into the cell, where these agents may cause irreversible structural and metabolic damage resulting in the death of the organism.

4.1.3 Mutagenesis

Since the very reactive oxygen free radicals can oxidatively damage or modify DNA, attention has recently been focused on the possible mutagenic nature of oxygen, especially under increased partial pressure (Bruyninckx *et al*, 1978). It has been known for some time that hyperbaric oxygen is mutagenic to *E.coli* (Fenn *et al*, 1957; Yost &

Fridovich, 1976) and several investigators studied the mutagenic action of oxygen and oxygen free radicals in organisms used in the Ames test (Bruyninckx *et al*, 1978; Moody & Hassan, 1982). Both base pair substitution and frame shift mutations are caused by the fluxes of superoxide radicals generated by activated phagocytes (Weitzman & Stossel, 1981) and paraquat in the presence of oxygen (Moody & Hassan, 1982) and are responsible for the highly mutagenic action of these agents in the Ames test.

Relative humidity influences the genetic stability of cultures since the removal of bound water from cellular constituents creates free radical lesions in these structures and accentuates their sensitivity to oxygen (Cox *et al*, 1971; Swartz, 1971); oxygen can react with these free radical lesions and increase or modify the damage to such an extent that mutations are caused (Benbough, 1969). If *Saccharomyces cerevisiae* cultures are freeze-, vacuum or air-dried in the presence of oxygen, there is an increase in the number of adenine-requiring colonies in the relative humidity range 0 to 53%, with the greatest number of mutants induced at 33% relative humidity.

4.1.4 The effect of oxygen on radiation damage

Oxygen modifies the effect of both X-rays (Thoday & Read, 1947; Hollaender *et al*, 1951; Howard-Flanders & Alper, 1957; Samuni *et al*, 1978) and gamma-rays (Van Hemmen *et al*, 1978) on bacterial cells and the killing of cells by ionizing radiation at ambient temperatures can be enhanced by a factor of 2 to 3 if oxygen is present during irradiation (Achey & Whitfield, 1968; Lehnert & Moroson, 1971). The extent of oxygen sensitization on radiation-induced damage is expressed as the oxygen enhancement ratio (OER) which is a quotient of doses under anaerobic and aerobic conditions required to reach a given level of damage (Samuni *et al*, 1978). *B.fragilis* is the only organism reported so far in which oxygen sensitizes the cells to far-UV irradiation and Jones *et al* (1980) reported an OER of between 1,15 and 1,45 for irradiated exponential phase *B.fragilis* cells. Since there are only quantitative and not qualitative differences between the production of lesions in DNA and induction of radioresistance by far-UV radiation and by ionizing radiation (Lydersen & Pollard, 1975; Kesavan *et al*, 1978), a knowledge of the oxygen sensitization of X-ray damage in cells will contribute to a better understanding of the oxygen effect on far-UV radiation damage in *B.fragilis*.

X-ray and UV radiation can inactivate cells by two different mechanisms, "indirectly" via radicals induced in the suspending medium and "directly" by lesions induced in cellular constituents by direct absorption of photons of radiation energy. The relative importance of the "direct" and "indirect" actions of UV radiation changes as a function of wavelength, with the indirect action becoming more important with the longer wavelengths. Oxygen possesses both a free radical and an electron affinic nature (Johansen & Howard-Flanders, 1965) and the major part of the oxygen sensitization of X-ray radiation damage results from the interaction between the electron affinic nature of oxygen and the products of the "direct" and "indirect" action of X-irradiation (Sapora *et al*, 1977b; Fielden *et al*, 1978).

4.1.4.1 Indirect action

Water radicals (hydrogen radical, hydroxyl radical, and the aqueous electron) are produced in the suspending medium by the action of X- and UV radiation. These radicals may diffuse into the cell and attack biologically important molecules. Oxygen can react rapidly with hydrogen radicals and aqueous electrons (Brustad, 1966) and converts them into the less active superoxide radicals. These superoxide

radicals in turn are known to produce single-strand breaks in the DNA, and Misra and Fridovich (1976) suggested that this was the real cause of the increased damage due to oxygen. Oxygen can also increase the effective yield of the deleterious oxidizing hydroxyl radicals by a process of electron sequestration: by reacting rapidly with aqueous electrons, it may scavenge this reducing species and so lessen the probability of hydroxyl radicals being reduced to hydroxide ions (Willson, 1981). The number of initial radical-induced strand breaks due to X- and UV radiation is thus effectively modified in the presence of oxygen.

4.1.4.2 Direct action

In the "direct" action X-ray and far-UV radiation energy is released directly within or very close to a functional unit. Absorption of a photon of radiation energy by a hydrogen atom in this unit can dislodge one of its electrons and so ionizes the hydrogen atom (Upton, 1982), creating a free radical lesion in the target molecule. Oxygen can react in two ways with these lesions:

(a) It can remove the ionized hydrogen atom from the damaged DNA, and form the very damaging peroxy radical which acts as a slow component

of the oxygen sensitization effect and produce "latent breaks" in the DNA (Ehret *et al*, 1960); Tallentire & Powers, 1963);

(b) Oxygen can also act as a "trap" for electrons liberated from the X-irradiated DNA by virtue of its high electron affinic nature. This will "fix" the DNA damage and prevent any possible restitution or self-repair by ion recombination (Alper, 1956; Howard-Flanders, 1958; Adams & Cooke, 1969; Samuni *et al*, 1978). "Fixed" damage does not involve the induction of a break or an alkali-labile site in the DNA but entails the creation of a site in the DNA which is susceptible to endonuclease action. The nature of the radiation-induced lesions is thus modified and this will affect the amount of repair that can be done by repair systems present in organisms.

The electron affinic radiosensitizer, para-nitroacetophenone (PNAP), can mimic the oxygen effect by increasing the yield of single-strand breaks in *E. coli* B/r DNA X-irradiated under anaerobic conditions (Sapora *et al*, 1975). However, PNAP is always less sensitizing than oxygen and the "repairable damage" profile with PNAP differs from the oxygen-sensitive repair profile in that no fixation of damage occurs

with PNAP (Sapora *et al*, 1977; Fielden *et al*, 1978). PNAP sensitization yielded five times less enzymatically produced breaks compared with aerobic X-irradiation. Ionizing radiation-induced single-strand breaks and fixed damage in *E.coli* both show an OER of 3,6 but there is a ratio of 1,3 enzymatically induced break to each X-ray radiation-induced break (Fielden *et al*, 1978).

4.1.5 The effect of oxygen on ionizing radiation damage in bacteriophages

Bacteriophages are often used in studies on the genetic mechanisms involved in repair because they are nuclear structures in which the radiation damage remains unmodified, in contrast with bacterial cells where efficient repair might continuously occur (Sapora *et al*, 1977b). A great deal of controversy existed as to whether oxygen can enhance the effect of ionizing radiation on phages or not. Srivastava (1974) claimed that the oxygen sensitization of ionizing radiation damage in phages was negligible, but a definite oxygen enhancement of X-irradiation damage was reported for phages irradiated within the host cell (Johansen *et al*, 1974 and 1975) or irradiated in the presence of radioprotectors (Van Hemmen *et al*, 1974; Srivastava, 1976). Samuni *et al* (1978) resolved this controversy by studying the effect of molecular oxygen and superoxide radicals on gamma

radiation-induced damage in T4 bacteriophages. They controlled the spectrum of water radicals produced in the suspending phosphate buffer by the addition of radical scavengers, as well as by a careful choice of the appropriate saturating gas. The damage induced in T4 phages by gamma radiation can be divided into exogenous and endogenous damage (Samuni *et al*, 1978).

4.1.5.1 Exogenous damage

In dilute aerobic buffer systems gamma radiation induced water radicals in the bulk system. These exogenous (outside the phage) radicals are responsible for 90% of the ionizing radiation damage observed in phages and primarily entails damage to phage proteins (Watson, 1952) since it is unlikely that these radicals will penetrate the phage coat to react with viral DNA. Both Alper (1954) and Misra & Fridovich (1976) believed that the superoxide radicals produced in the suspending medium by the reaction between the radiation-induced hydrogen radicals and aqueous electrons and molecular oxygen, play a major role in inactivation of phages. Samuni *et al* (1978), however, showed that the primary hydroxyl radicals are the reactive species and neither superoxide radicals nor hydrogen peroxide radicals are involved in the inactivation of phages. No oxygen enhancement of ionizing radiation damage is thus

observed for phage suspensions irradiated in the absence of radical scavengers.

4.1.5.2 Endogenous damage

In the presence of radical scavengers (or inside a host bacterium) the small portion (10%) of gamma radiation damage initiated inside the phage by "direct" radiation action, is observable. This endogenous damage is usually masked in dilute systems by the major effect of the exogenous damage. Oxygen can react directly with the damaged target molecule in the phage, presumably the viral proteins rather than viral DNA, causing irreversible peroxidation of the primary lesions and suppression of any restitution process (Samuni *et al*, 1978). This reaction may explain the oxygen enhancement of ionizing radiation damage in phages observed by several investigators (Taylor & Ginoza, 1967; Boyce & Tepper, 1968; Freifelder, 1968; Johansen *et al*, 1971 and 1974).

4.1.6 Radioprotectors

Several compounds can protect organisms against the effect of ionizing and UV radiation because of their free radical scavenging ability. A very interesting finding, however, is that a few chemicals which act

as radiosensitizers under anaerobic conditions, act as radioprotectors under aerobic conditions.

Caffeine is a well known radiosensitizer which potentiates UV and X-ray-induced damage in a wide range of anaerobic metabolizing systems (Harm, 1967; Fabre, 1972; Witte & Böhme, 1972; Ahnström, 1974) mainly by inhibiting excision repair of radiation damage (Rupert & Harm, 1966; Setlow, 1967; Swenson, 1976). In aerobic systems, however, caffeine can protect against oxygen-dependent X-ray and gamma-ray radiation damage (Kesavan *et al*, 1973; Ahnström, 1974; Kesavan & Ahmad, 1974; Kesavan & Afzal, 1975; Nadkarni & Kesavan, 1975; Kesavan & Ahmad, 1976; Kesavan & Dodd, 1976; Kesavan *et al*, 1978). Caffeine restitutes the oxygen-sensitive radiation-induced lesions in the DNA by a process of hydrogen donation. This decreases the possibility of fixation of damage by oxygen. The same protective action of caffeine has not as yet been reported for far-UV radiation under aerobic conditions.

The radioprotector N-ethylmaleimide (NEM) and the chemicals potassium permanganate, potassium iodide, potassium nitrate and potassium ferrocyanide, react in a similar manner to caffeine in that they all potentiate anaerobic ionizing radiation damage and protect against oxygen sensitization of ionizing

radiation-induced damage in aerobic systems (Kesavan *et al*, 1973; Sharma & Kesavan, 1975).

4.1.7 Repair of oxygen-dependent damage

Although it is known that oxygen and oxygen free radicals can damage and modify DNA, it is not so clear to what extent such oxidative damage or modifications of DNA is repairable. The discovery of inducible radical scavenging enzymes (superoxide dismutases and catalases/peroxidases) has drawn the attention away from possible DNA repair processes involved in the survival of anaerobes in the presence of oxygen.

Several studies have been done on the repair of oxygen-dependent ionizing radiation damage in DNA. Unlike oxidative damage, oxygen-dependent radiation damage in DNA is caused not only by the free radical action of oxygen, but also involves modification of radiation-induced damage by the electron affinic action of molecular oxygen.

4.1.7.1 Repair of oxidative damage

Although the nature of the DNA damage induced by the various oxygen free radicals is well known, the only mechanism of repair of oxidative damage reported

so far involves the single-strand breaks induced in DNA by the stable oxygen radical, hydrogen peroxide. Ananthaswamy & Eisenstark (1977) reported that the repair of hydrogen peroxide-induced DNA breaks requires both the *polA*$^+$ and the *recA*$^+$ pathways, but Carlsson & Carpenter (1980) claimed that the *recA*$^+$ gene product is the more important factor in the protection of DNA against hydrogen peroxide toxicity. Hartman & Eisenstark (1978) divided the damage done by hydrogen peroxide into *recA* -repairable (at low hydrogen peroxide concentrations) and *recA* -irrepairable damage (at high concentrations of hydrogen peroxide).

Support for the conclusion that both the *pol*$^+$ and the *rec*$^+$ pathways are involved in repair of oxidative damages, comes from the results of Gross *et al* (1971) and Morimyo (1982). These investigators found that although the *E.coli* single mutants *polA* and *recB* are viable under aerobic conditions, the double mutant *polA recB* of *E.coli* is inviable in the presence of oxygen. The double mutant shows an enhanced colony formation under anaerobic conditions (Morimyo, 1982), or when it is aerobically plated with chloroform-killed *E.coli* cells or sonic extracts of *E.coli* cells which possess respiratory activity (and therefore can scavenge oxygen from the plating medium)(Monk & Kinross, 1972; Morimyo, 1982). Morimyo (1982) suggested that

the presence of both the *polA*$^+$ and the *recB*$^+$ gene products is needed for normal growth of aerobes, since these proteins are responsible for repairing DNA damage sustained during respiration.

4.1.7.2 Repair of oxygen-dependent ionizing radiation damage

4.1.7.2.1 Timescale

Many investigators agree that oxygen sensitization of ionizing radiation damage has at least two components. Class II damage is dependent on oxygen during irradiation (Davies, 1968; Webb, 1970; Weiss & Santomasso, 1981), while class III damage is dependent on the presence of oxygen after irradiation (Powers *et al*, 1960; Weiss & Santomasso, 1981). The class II damage is consistent with the interaction of oxygen with the transient free radical DNA lesions produced during irradiation (Howard-Flanders & Moore, 1958; Ehret *et al*, 1960; Adams *et al*, 1968; Brustad, 1968; Epp *et al*, 1973; Michaels *et al*, 1973), and Michaels *et al* (1981) placed the timescale for such an oxygen sensitization reaction in the microsecond to millisecond range. Shenoy *et al* (1975) reported that the exposure of *Serratia marcescens* cells to oxygen a few milliseconds before X-irradiation is sufficient to produce the full oxygen effect, whereas exposure to oxygen 5 to 10 milliseconds after irradiation

did not affect the anaerobic response.

The effect of adding oxygen to bacteria either before or after X-irradiation was found to depend on whether the bacteria are irradiated in broth or in a buffered solution. A large portion of the radiation damaging effect in buffer or broth is due to water radicals produced in the suspending medium immediately outside the cell membrane or radicals formed directly in the membrane itself. In broth the exogenous radicals are rapidly scavenged by organic materials in the medium and damage produced in the membrane can also be chemically repaired by hydrogen atom donation by extracellular organic material. The scarcity of efficient hydrogen donors in buffer solutions causes the membrane-damaging free radicals in this system to be relatively long lived and these radicals are thus available for reaction with oxygen added several milliseconds after irradiation (Shenoy *et al*, 1975). The results of Shenoy *et al* (1975) confirmed that bacteria are more sensitive to the oxygen effect when X-irradiated in buffer than in nutrient broth and that the pre-irradiation contact times required to produce the full oxygen enhancement are significantly larger in broth than in buffer.

4.1.7.2.2 Enzymatic repair

More than one repair process occurs within the first five minutes after X-irradiation and the disadvantage of conventional techniques in elucidating possible early events resulted in the development of the gas explosion technique (Michaels *et al*, 1973), rapid liquid mixing process (Shenoy *et al*, 1975), rapid lysis technique (Fox *et al*, 1976) and the double pulse method (Ling *et al*, 1978), to experimentally record times shorter than 10 μseconds. Many of the rates of X-radiation-induced single-strand breakage in the presence of oxygen previously reported for *E.coli* were erroneously low because the existence of the polymerase repair system which can rejoin breaks in buffer at room temperature in a very short time, was not recognised (Town *et al*, 1972).

Oxygen has no effect on the amount of damage produced during X-irradiation (Boye *et al*, 1974; Sapora *et al*, 1975) or UV irradiation (Jones *et al*, 1980) and Town *et al* (1973) proposed the working hypothesis of "equal breaks, different repair" which can account for the greater sensitivity of cells X-irradiated under aerobic than under anaerobic conditions. They observed three different types of enzymatic repair of single-strand breaks in the DNA of X-irradiated cells: an ultrafast

repair, a second type which acts at 2 to 5 minutes after irradiation at room temperature, and a third medium-dependent type which needs 40 to 80 minutes at 37°C.

(a) Ultrafast repair

The ligase enzyme is responsible for this temperature-independent ultrafast repair of single-strand breaks (Town *et al*, 1972 and 1973). Direct sealing of breaks can occur within seconds even at very low temperatures. The ultrafast repair system preferentially operates under anaerobic conditions (Town *et al*, 1972; Srivastava, 1976; Samuni *et al*, 1978) and Town *et al* (1973) found that this repair system could repair 75% of the X-ray-induced breaks produced under anaerobic conditions, but only 23% of the breaks produced under aerobic conditions. Town *et al* (1972) postulated that oxygen modifies the DNA breaks so that they are no longer a substrate for the ultrafast repair system.

(b) Polymerase-dependent repair

The strand repair occurring after about 2

seconds in an aerobic X-irradiated system is affected by temperature and is attributable to the action of the polymerase enzyme(s)(Town *et al*, 1973; Boye *et al*, 1974; Sapora *et al*, 1975). The optimum temperature for this type of repair ranges between 25° and 37°C. The presence of oxygen during X-irradiation appears to induce more efficient repair of X-ray-induced breaks, as compared with repair of breaks under anaerobic conditions (Hall, 1972; Koch & Painter, 1975), and this may be linked to the conversion of transient free radical DNA lesions into more stable DNA lesions by oxygen fixation of the damage (Sapora *et al*, 1975). These stable lesions can then be recognized by polymerase-associated endonucleases which produce one single-strand break for each damaged site. Polymerase I is capable of repairing all enzymatically induced single-strand breaks, but only 80% of the radiation-induced alkali-labile breaks in *E.coli* B/r, and polymerase III-dependent repair of X-ray damage only occurs when the X-irradiated cells are held in the presence of of oxygen (Sapora *et al*, 1977b).

(c) Medium-dependent repair

The slow timescale and the medium- and temperature-dependency of the type III repair of X-ray-induced damage (Town *et al*, 1973; Sapora *et al*, 1975) are consistent with the induction of a repair system (e.g. the *rec*$^+$ repair system). Both the *pol*$^+$ and the *rec*$^+$ gene products can repair discontinuities or gaps in the DNA (Okazaki *et al*, 1971; Monk & Kinross, 1972) and several investigators claim that both functions are important for the repair of single-strand breaks induced by X-rays (Morimyo *et al*, 1968; Kapp & Smith, 1970; Town *et al*, 1971) and UV radiation (Rupp & Howard-flanders, 1968; Smith & Meun, 1970; Monk *et al*, 1971). These two functions also appear to be responsible for the major part of the repair of oxygen-dependent X-radiation damage: if the drug quinacrine, which inhibits both *rec* repair (Fuks & Smith, 1971) and *pol* repair (Town *et al*, 1972) in *E.coli*, is added to X-irradiated *E.coli* cells, the repair of aerobic breaks is inhibited almost completely (Town *et al*, 1972). Very few reports exist on the importance of the excision repair system for the repair of oxygen-dependent X-ray-induced damage. The results of Sapora

et al (1977a), however, indicated that an *uvrA*$^-$ *E.coli* strain is only slightly more sensitive to X-rays under aerobic conditions than the wild type *E.coli* strain.

4.1.8 Most important biological target for oxygen damage: theories

It is not unlikely that living systems, in their enormous complexity, contain multiple critical targets for oxygen and oxygen radicals. Alper (1968) also proposed that there are two cellular sites where oxygen enhances radiation damage: type O oxygen damage occurs in cell membranes and type N oxygen damage involves the sensitization of nucleic acid radiation damage.

4.1.8.1 DNA

The DNA of organisms have repeatedly been shown to be the target for the damaging action of oxygen free radicals (Ananthaswamy & Eisenstark, 1977; Bruyninckx *et al*, 1978; Cadet & Teoule, 1978; Lynch & Fridovich, 1978; Moody & Hassan, 1982). This damage ranges from simple single-strand breaks to liberation of all four DNA bases and includes base pair substitution and frame shift mutations. Oxygen can also interfere with the efficient repair of ionizing radiation damage to DNA and many investigators ascribed the greater lethality

after ionizing radiation in the presence of oxygen (than under anaerobic conditions) to irrepairable fixation of DNA damage by molecular oxygen (Sapora *et al*, 1977b; Fielden *et al*, 1978; Samuni *et al*, 1978). Considerable evidence is accumulating, however, suggesting that DNA damage is not solely responsible for the overall biological effect of oxygen sensitivity (Shenoy *et al*, 1975).

4.1.8.2 Membranes

Cell membranes have been proposed as possible sites where lethal radiation and oxygen damage could occur, independently of or by interaction with nucleic acid damage (Alper, 1968; Myers, 1970; Shenoy *et al*, 1975). Ionizing and UV radiation affect cell membranes in such a way that cells become more permeable (Bruce, 1958; Harrison *et al*, 1958), leading to the consequent release of intracellular material such as potassium ions and ATP (and sometimes other nucleotides) into the suspending medium at a dose-dependent rate (Harrison *et al*, 1958; Merrick & Bruce, 1965; Billen, 1967). The loss of ATP from damaged bacteria presumably causes an uncoupling of metabolism associated with active transport, and damage to cell membranes is often evaluated by the passive efflux of intracellular potassium ions at $0^{\circ}C$ (Kesavan, 1973;

Cancelliere *et al*, 1975; Sharma & Kesavan, 1975). A large increase in potassium ion release from the membranes of X- and UV-irradiated yeast cells was observed in the presence of oxygen (Bruce, 1958) and Alper (1968) reported that radiation damage to membranes (type O damage) has 4 to 5 times the probability of becoming lethal when oxygen is present during X- and UV irradiation.

The greater solubility of oxygen in the lipophilic cell membrane (Ewing, 1981) and the extremely damaging peroxidative action of the oxygen free radicals on cell membrane components were discussed in section 4.1.1. Evidence of specific oxygen damage was found in membranes of frozen and/or lyophilized bacteria and involves changes in the membrane-associated DNA initiation complex and in the membrane permeability (Israeli *et al*, 1975). Halliwell (1982) reported that the peroxidation of membrane lipids by superoxide radicals results in increased leakiness and decreased membrane fluidity and Cancelliere *et al* (1975) found that the structural abnormalities in membranes of *E.coli* K12 cells exposed to fluxes of superoxide radicals (as observed under the electron microscope) correlate well with a loss of viability under these conditions.

4.1.8.3 Enzymes

The free radical inactivation of many enzymes *in vivo* and *in vitro* have been extensively studied (Collinson *et al*, 1950; Brustad, 1966; Adams *et al*, 1971; Masuda *et al*, 1971; Adams & Redpath, 1974) and the radiation sensitivity of a number of enzymes increases by a factor of 2 to 3 in the presence of oxygen (Alexander, 1957; Shalek & Gillespie, 1959; Hutchinson, 1961). The hydroxyl radical was generally found to be the active radical species in enzyme inactivation (Michel, 1975).

The hypothesis of enzyme inactivation is an explanation for oxygen toxicity did not previously gain wider acceptance because oxygen-mediated enzyme inactivation *in vitro* appeared to be too slow to account for the symptoms of oxygen poisoning that occurred in the intact organism and there was no conclusive evidence for the inactivation of an enzyme by oxygen itself (Fee, 1982). However, several examples of enzymes which are rapidly and directly inactivated by dioxygen are now known. Halliwell (1982) reported that enzyme inactivation may account for the rapid growth inhibiting effect of pure oxygen at high pressure in *E.coli*. The enzymes amidophosphoribosyl transferase, involved

in purine biosynthesis (Itakura & Holmes, 1979), an dihydroxyacid dehydratase involved in branched chain amino acid biosynthesis, are both inactivated by oxygen *in vivo*, even in the presence of superoxide dismutase enzymes (Fee, 1982). Since the inactivation of a critical enzyme can disrupt the overall metabolism of the cell (Haugaard, 1968), it will be essential in understanding oxygen toxicity to identify and study sensitive enzymes and their reactivity towards oxygen (Fee, 1982).

B.fragilis strain Bf-2 is interesting in that although it is an obligate anaerobe it can be maintained in various aerobic holding solutions for 1 to 6 hours without loss of viability (Jones & Woods, 1981; Slade *et al*, 1981) and there is some evidence that the cells are able to carry out DNA repair processes in the presence of oxygen and its free radical, hydrogen peroxide (Slade *et al*, 1983a and 1983b). However, *B.fragilis* differs from other bacteria in that its inactivation by far-UV light is dependent on the presence of molecular oxygen (Jones *et al*, 1980; Slade *et al*, 1981); the cells are more sensitive to far-UV irradiation under aerobic conditions than under anaerobic conditions. In contrast with this

sensitization effect, pretreatment of cells with hydrogen peroxide under anaerobic conditions enhances the survival of UV-irradiated *B.fragilis* cells (Slade *et al*, 1983b). Since no studies on the effect of both oxygen (and its free radicals) and UV irradiation on macromolecular synthesis in *B.fragilis* have been reported, we investigated the effects of oxygen and hydrogen peroxide on colony formation and on nucleic acid and protein synthesis in unirradiated and UV-irradiated *B.fragilis* cells.

4.2 MATERIALS AND METHODS

4.2.1 Bacteria and media

The *B.fragilis* Bf-2 strain which has been described in section 2.2.1, was used in these studies. Brain heart infusion broth and agar, supplemented with hemin, menadione, and cysteine (Holdeman & Moore, 1972) were used for bacterial propagation at 37^{o}C. Prereduced one-quarter-strength Ringer solution was used as a dilution buffer (Jones & Woods, 1981). Irradiation and radioactive labelling of the cells were carried out in a defined minimal medium (Varel & Bryant, 1974).

4.2.2 Anaerobic and aerobic treatments

All manipulations and treatments requiring anaerobic conditions were carried out in an anaerobic glove cabinet (Forma Scientific, Marietta, Ohio) at 37°C in an atmosphere of 70% N_2, 20% CO_2 and 10% H_2. Cultures were aerated in an aerobic incubator at 37°C by either shaking 4 ml samples in 14 ml glass centrifuge tubes (tube method) or by adding small samples (0,1 ml per well) in a microtitre plate which was incubated without agitation (microtitre plate method). The redox indicator, resazurin, in the medium remained pink during the aerobic treatments but was colourless under anaerobic conditions. In experiments where the aerobic cultures were restored to anaerobic conditions, the resazurin indicator turned colourless within approximately 3 min after introducing the cultures into the anaerobic cabinet.

4.2.3 DNA, RNA and protein synthesis

DNA synthesis was determined by the incorporation of [2-^{14}C] thymidine (4 µCi ml^{-1}), RNA synthesis by the incorporation of [5,6-^{3}H]uracil (15 µCi ml^{-1}), and protein synthesis by the incorporation of [^{35}S] methionine (20 µCi ml^{-1}) into trichloracetic acid (TCA) precipitable material. The labelled chemicals

were supplied by The Radiochemical Centre, Amersham, England, and the final concentrations of thymidine, uracil and methionine were 7, 15 and 25 μg ml^{-1} respectively, which were saturating for both uptake and incorporation over 240 min.

The effects of UV irradiation and oxygen on DNA, RNA and protein synthesis were determined in prelabelled and unlabelled cells (Smith & O'Leary, 1968; Setlow & Setlow, 1970). Overnight brain heart infusion broth cultures were innoculated into minimal medium and incubated until the cultures were in exponential growth and reached a turbidity of 0,15 at 600 nm. The label was added and the cultures incubated for a further 60 min (turbidity of 0,2 at 600 nm, approximately 1,5 x 10^8 colony-forming units ml^{-1}) prior to irradiation and exposure to oxygen (prelabelled cells). After irradiation and/or exposure to oxygen the cells were reincubated in the presence of the label. In experiments with unlabelled cells, the cells were irradiated, exposed to oxygen and the label added immediately before reincubation in the presence of the label.

The effects of UV irradiation and/or hydrogen peroxide on DNA synthesis under anaerobic and aerobic conditions were determined in prelabelled cells. Samples (4 ml) of unirradiated and UV-irradiated prelabelled cells

were treated with different concentrations of hydrogen peroxide (between 0,0001 and 0,1%, vol/vol) under anaerobic conditions, or hydrogen peroxide was added to the prelabelled unirradiated cells immediately after exposure to oxygen.

4.2.4 UV irradiation

The effect of UV radiation on oxygen- and hydrogen peroxide-treated cells was determined. Samples (9 ml) of exponential phase cultures (turbidity of 0,2 at 600 nm) were irradiated in open glass petri dishes with a Fluotest Piccolo Hanau Quartz germicidal lamp (254nm). The dose rate was measured with a Blak-Ray UV meter (model J-225; UV Products Inc, San Gabriel, Calif.) and samples were irradiated at a dose rate of 1,0 J $m^{-2}s^{-1}$. In experiments where the effect of UV and oxygen on macromolecular synthesis was studied, the exponential phase cultures were exposed to oxygen for a few seconds before irradiation in the presence of oxygen. Irradiation was not carried out in the presence of hydrogen peroxide, but the hydrogen peroxide was added to the cultures immediately after UV irradiation. Survival curves of cells irradiated with increasing fluences were determined, and labelling experiments were carried out with cultures which were irradiated aerobically to different survival levels.

4.2.5 Effect of oxygen on uptake of radioactive thymidine

The rate of uptake of [^{14}C] thymidine was determined in unlabelled cells before exposure to air and after 30 and 100 min exposure to air in the microtitre plate method. The label was added to the sample and uptake by the cells was estimated over 20 min by filtration of 100 µl aliquots and washing with minimal medium on 0,45 µm (pore size) membrane filters.

4.2.6 DNA turnover

DNA turnover experiments were carried out on *B. fragilis* cultures exposed to oxygen for prolonged periods of time. An exponential phase culture with a turbidity of 0,15 at 600 nm was divided into two. The one culture was prelabelled as previously described and aerated by the microtitre plate method when it reached a turbidity of 0,2 at 600 nm. The other culture was treated in the same way except that the prelabelling step was omitted. After 85 min of aeration the prelabelled cells were harvested by centrifugation, resuspended in fresh prewarmed minimal medium without label and reincubated under aerobic conditions for a further 80 min. [^{14}C] Thymidine (4 µCi ml^{-1}) was added to the unlabelled culture at 85 min and incubation continued under identical conditions as the prelabelled culture.

4.2.7 Effect of chloramphenicol and caffeine

The effects of chloramphenicol and caffeine were determined on DNA synthesis in unirradiated and UV-irradiated (1,3% survival) prelabelled cells exposed to oxygen. The minimal inhibitory concentrations of chloramphenicol and caffeine were 1 μg ml^{-1} and 2,5 mg ml^{-1}, respectively. Chloramphenicol (5 μg ml^{-1}) and caffeine (1 mg ml^{-1}) were added to the broth cultures immediately after irradiation and exposure to oxygen.

4.2.8 Effect of oxygen and hydrogen peroxide on colony formation

Exponential phase cells (turbidity of 0,2 at 600 nm) were exposed to oxygen by the microtitre plate method, or treated with hydrogen peroxide (0,0001 and 0,01%, v/v) under anaerobic conditions at 37°C. Colony formation was determined after different time intervals by plating on brain heart infusion agar.

4.3 RESULTS

4.3.1 Effect of oxygen on DNA synthesis and colony formation

The effect of oxygen on DNA synthesis and viability in exponential phase *B. fragilis* cells grown in the minimal medium was determined. Aeration was carried out by

two methods. The tube method involved a relatively large sample (4 ml) from which smaller samples (0,1 ml) could be removed and assayed. The microtitre plate method involved a series of identical small samples (0,1 ml) and the entire sample was assayed at a particular sampling time. It was necessary to establish that both methods were comparable because only the microtitre plate method could be used after exposure to both UV radiation and oxygen. Exposure of *B.fragilis* cells to UV radiation and oxygen caused rapid visible clumping of the cells which made sampling difficult and inaccurate.

The growth (colony-forming units) of exponential *B.fragilis* cells in minimal medium was inhibited when the cells were exposed to oxygen by the tube and microtitre plate methods (Fig. 4.1). There was no decrease in the viability of the cells exposed to oxygen for 60 and 100 min in the tube and microtitre plate methods respectively. The viability of the cells decreased between 60-90 min in the microtitre plate method and between 100-120 min in the tube method. Decrease in viability occurred at a similar rate in the two aeration systems (decrease of 11 and 13 colony-forming units ml^{-1} 10 min^{-1}).

Exposure of the *B.fragilis* cells to oxygen caused

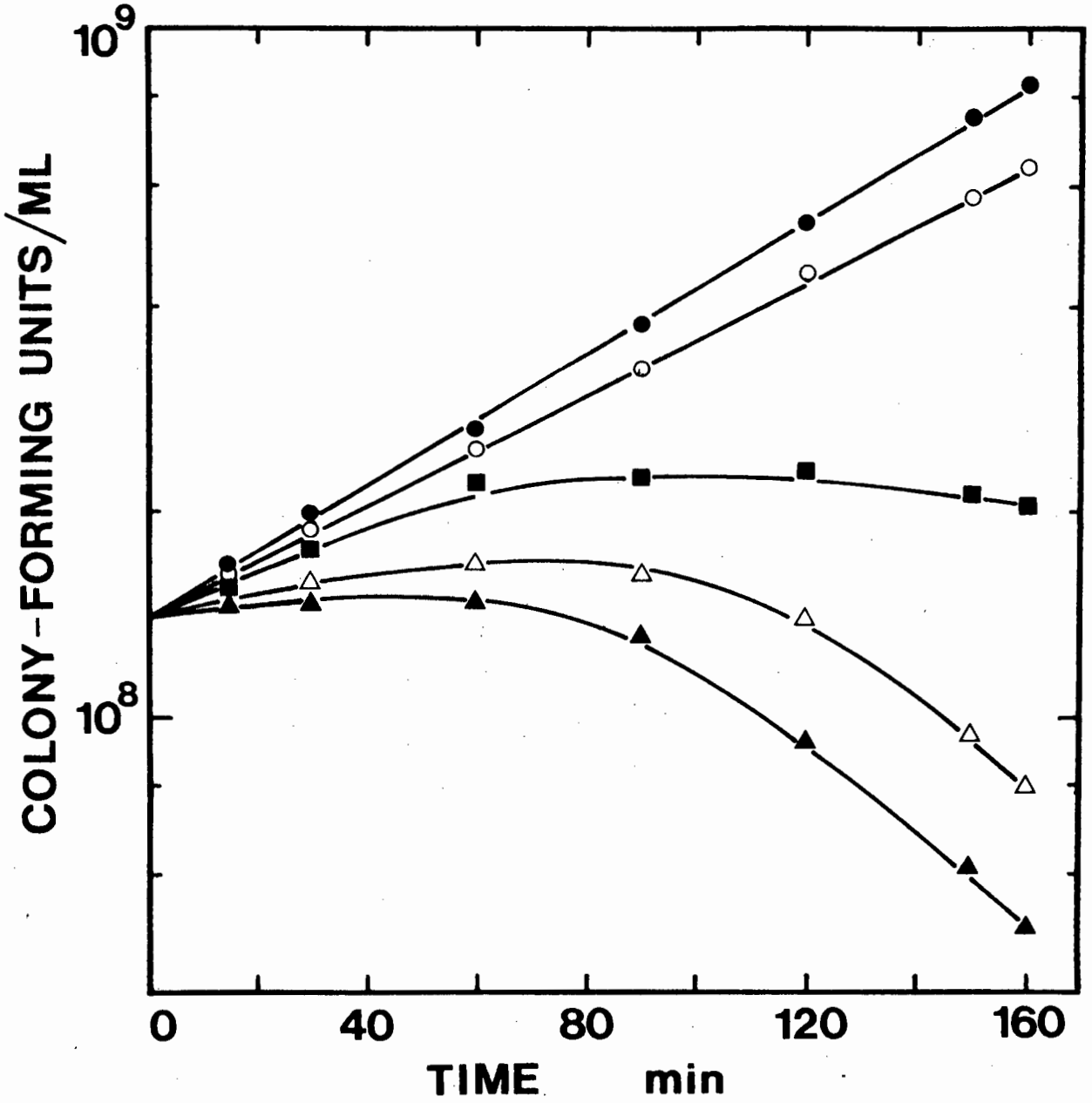

FIG 4.1 Effects of oxygen and hydrogen peroxide on the viability of *B.fragilis* cells. Anaerobic control (●); cells exposed to air by the tube method (△) and the microtitre method (▲); cells exposed to 0,001% (○) and 0,01% (■) hydrogen peroxide under anaerobic conditions.

reduction in DNA synthesis which was slower in the microtitre plate method than in the tube method (Fig 4.2). DNA synthesis was eventually inhibited by oxygen after 60 and 90-100 min incubation in the microtitre and tube methods respectively. In the two methods there was a correlation between the decrease in viability and the inhibition of DNA synthesis.

Prelabelled cultures which were restored to anaerobic conditions at time 30 min after exposure to oxygen by the microtitre plate method, resumed DNA synthesis after a short lag period at a rate comparable to the anaerobic control (Fig 4.3). Cultures restored to anaerobic conditions after 45 min of aeration had a longer lag period before resumption of DNA synthesis at an exponential rate similar to the anaerobic control rate. Cultures introduced into the anaerobic cabinet after 80 min of aeration, however, did not resume DNA synthesis after an additional 80 min of anaerobic incubation (Fig 4.3).

The rate of uptake of [^{14}C] thymidine in unexposed (anaerobic) cells was 300 cpm ml^{-1} min^{-1}, and 240 and 200 cpm ml^{-1} min^{-1} after 30 min and 100 min exposure to oxygen respectively. Uptake of label was reduced by oxygen but continued after inhibition

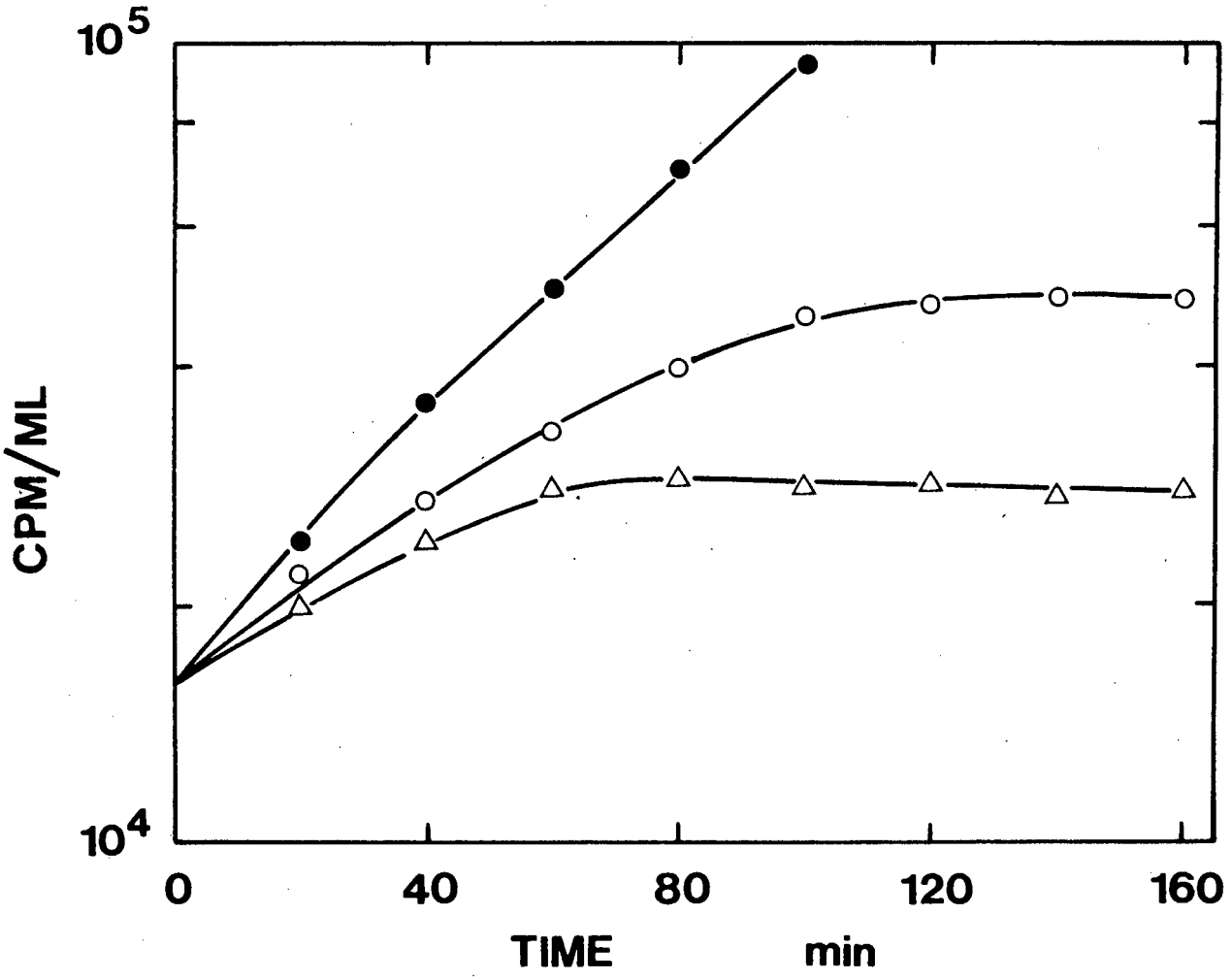

FIG 4.2 Effect of oxygen on DNA synthesis in prelabelled *B.fragilis* cells. The cells were labelled with [^{14}C] thymidine 60 min before exposure to oxygen by the tube method (○) and the microtitre plate method (△). Anaerobic control (●).

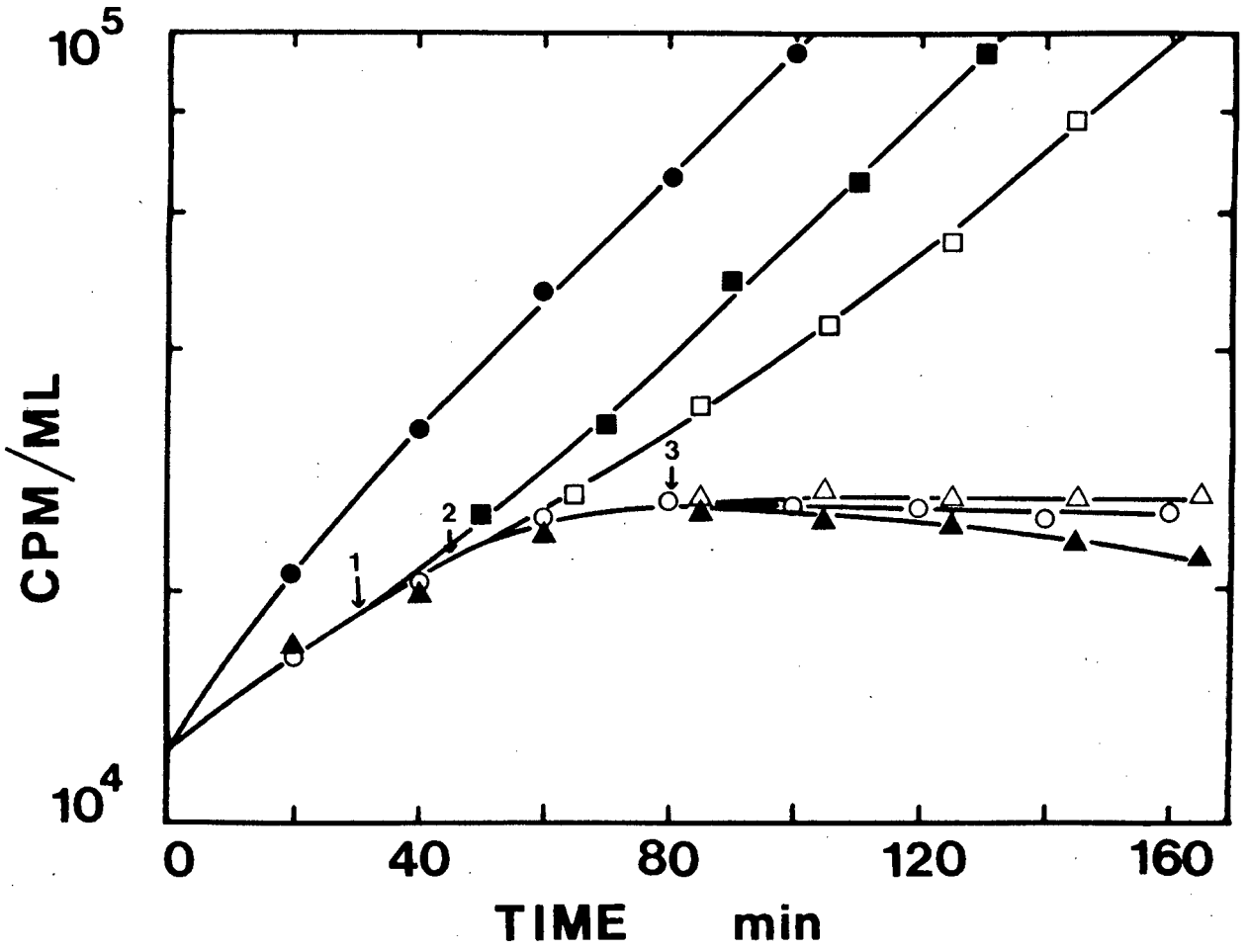

FIG 4.3 DNA turnover in an aerated culture and the effect of restoration to anaerobic conditions on DNA synthesis in prelabelled aerated *B.fragilis* cells. Anaerobic control (●); cells restored to anaerobic conditions at 30 min (↓1) (■), 45 min (↓2) (□) and 80 min (↓3) (○). Unlabelled cells exposed to oxygen for 85 min and then labelled (△); prelabelled cells exposed to oxygen for 85 min, harvested and resuspended in minimal medium without label (▲).

of DNA synthesis and we concluded that the inhibition of DNA synthesis after 60 min by oxygen was not due to a shutoff of isotope transport.

DNA turnover experiments were carried out to determine whether the observed plateau of incorporation of [^{14}C] thymidine into DNA was due to an inhibition of DNA synthesis, or reflected a steady state condition with equal rates of degradation and resynthesis of labelled DNA (Fig 4.3). No incorporation of label was observed after the addition of [^{14}C] thymidine to an unlabelled culture which had been exposed to oxygen for 85 min. There was a very slow loss of label from the trichloroacetic acid-precipitable cellular fraction in pre-labelled cultures after 85 min exposure to oxygen.

4.3.2 Effect of oxygen on RNA and protein synthesis

RNA and protein synthesis were also affected by oxygen and synthesis was decreased to a greater extent in the microtitre plate method than in the tube method (Figs 4.4 and 4.5). RNA and protein synthesis were less sensitive to oxygen than DNA synthesis. Since the incorporation of isotopes into RNA and protein continued after DNA synthesis was inhibited, the inhibition of DNA synthesis was not due to the inhibition of RNA or protein synthesis by oxygen.

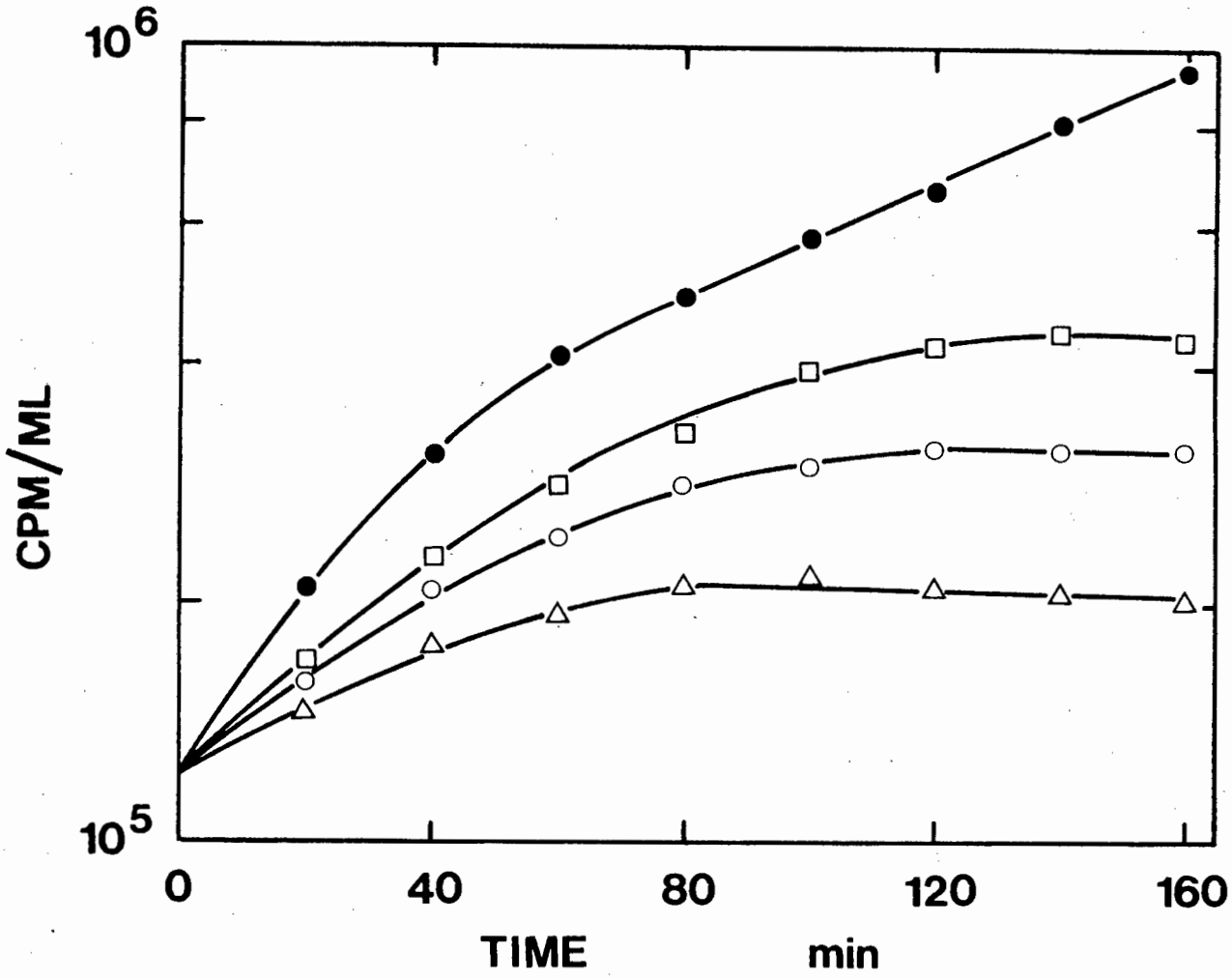

FIG 4.4 Effect of oxygen and UV irradiation on RNA synthesis in prelabelled *B.fragilis* cells. The cells were labelled with [^{3}H] uracil 60 min before exposure to oxygen and UV radiation. Unirradiated anaerobic control (●); cells exposed to oxygen by the tube method (□), and the microtitre plate method (○); cells exposed to oxygen by the microtitre plate method and irradiated (1% survival) (△).

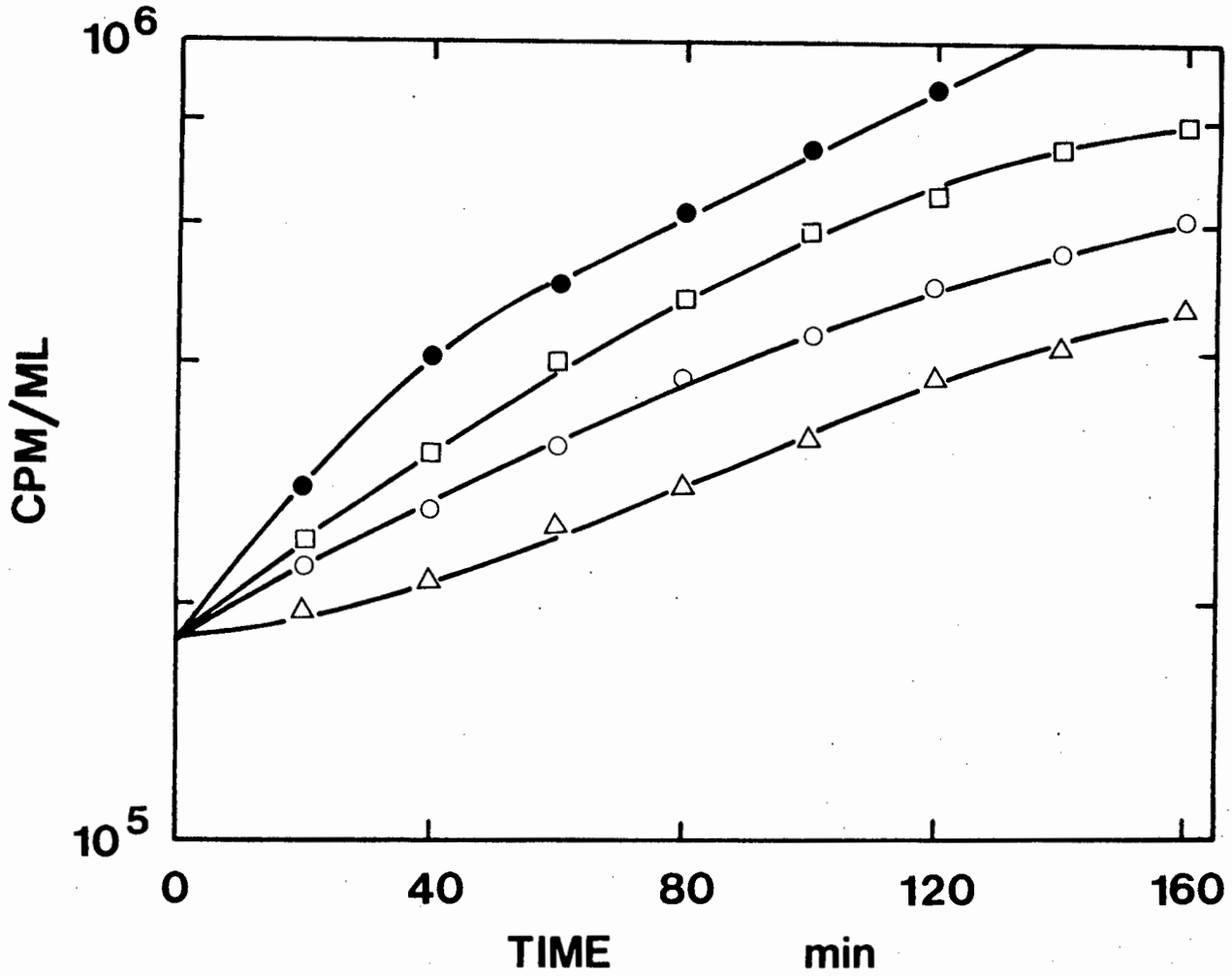

FIG 4.5 Effect of oxygen and UV radiation on protein synthesis in prelabelled *B.fragilis* cells. The cells were labelled with [^{35}S] methionine 60 min before exposure to oxygen and UV radiation. Unirradiated anaerobic control (●); cells exposed to oxygen by the tube method (□) and the microtitre plate method (○); cells exposed to oxygen by the microtitre plate method and irradiated (1% survival) (△).

4.3.3 Effect of UV radiation and oxygen on DNA synthesis

B.fragilis cells which were irradiated and held in minimal medium under aerobic conditions formed visible clumps within 1 hour after irradiation. In order to avoid errors due to inaccurate sampling, the microtitre plate method was used for all aerobic UV radiation experiments. Irradiation of prelabelled *B.fragilis* cells under anaerobic conditions resulted in the degradation of DNA (Fig 4.6). The degradation phase continued for 40 min before DNA synthesis was detected. Aerobic UV irradiation of *B.fragilis* prelabelled with [^{14}C] thymidine (1,8% survival) resulted in the complete inhibition of DNA synthesis (Fig 4.6). The addition of chloramphenicol or caffeine did not affect the pattern of DNA synthesis in prelabelled cells irradiated under aerobic conditions (Fig 4.6). DNA synthesis in unirradiated cells exposed to oxygen was not affected by chloramphenicol or caffeine.

DNA synthesis was also determined in unlabelled cells which were irradiated under aerobic conditions with increasing doses of UV light prior to the addition of [^{14}C] thymidine (Fig 4.7). The degree of inhibition of DNA synthesis in aerobically irradiated cultures was dose-dependent

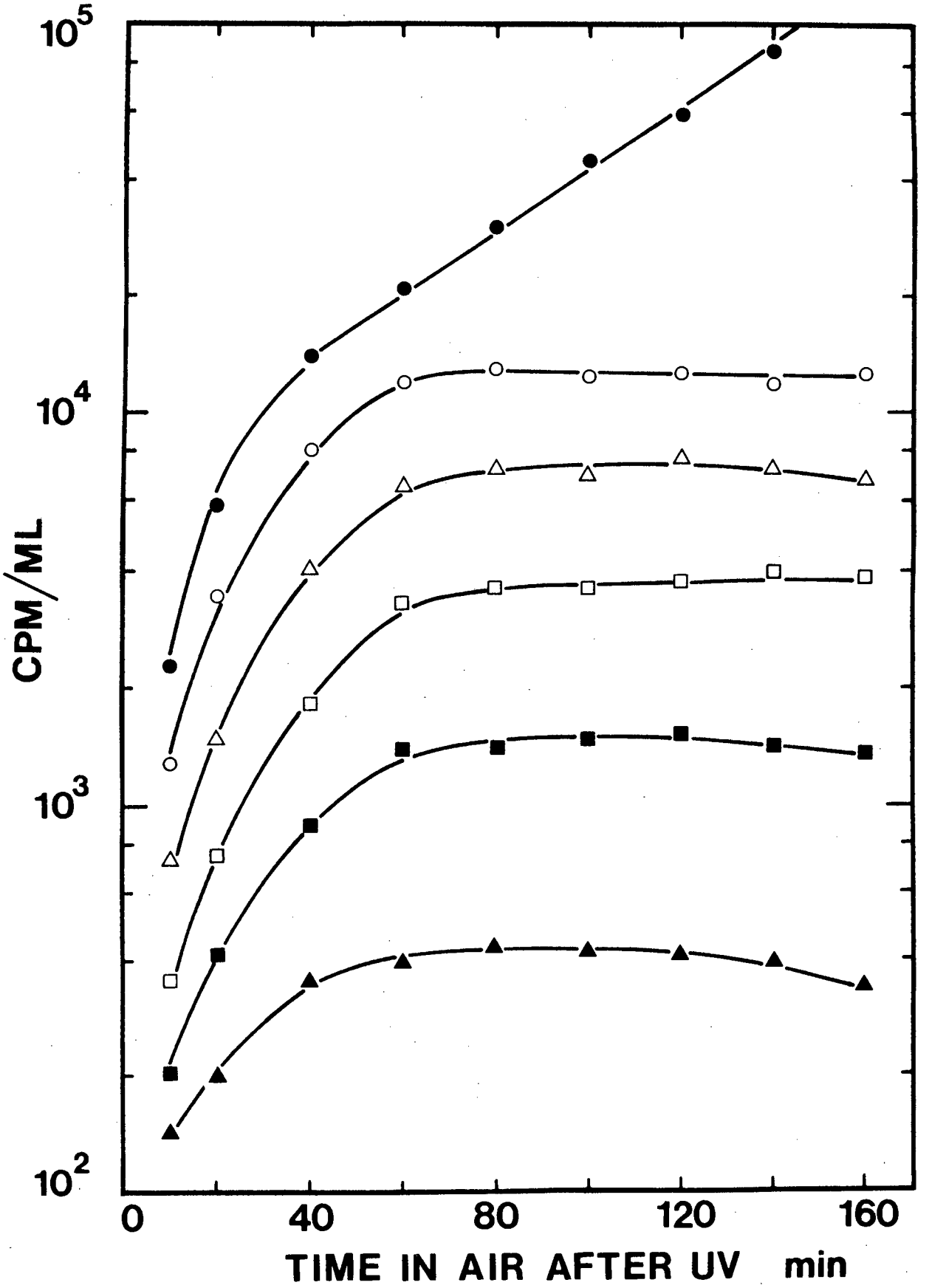

FIG 4.6 Effect of oxygen and increasing doses of UV radiation on DNA synthesis in unlabelled *B.fragilis* cells. Exponential phase cells were irradiated and labelled with [^{14}C] thymidine. Unirradiated anaerobic control (●). Cells exposed to oxygen (○) and irradiated with UV light (J m^{-2}): 20 (87% survival) (△), 40 (23% survival) (□), 60 (4,4% survival) (■) and 80 (0,3% survival) (▲).

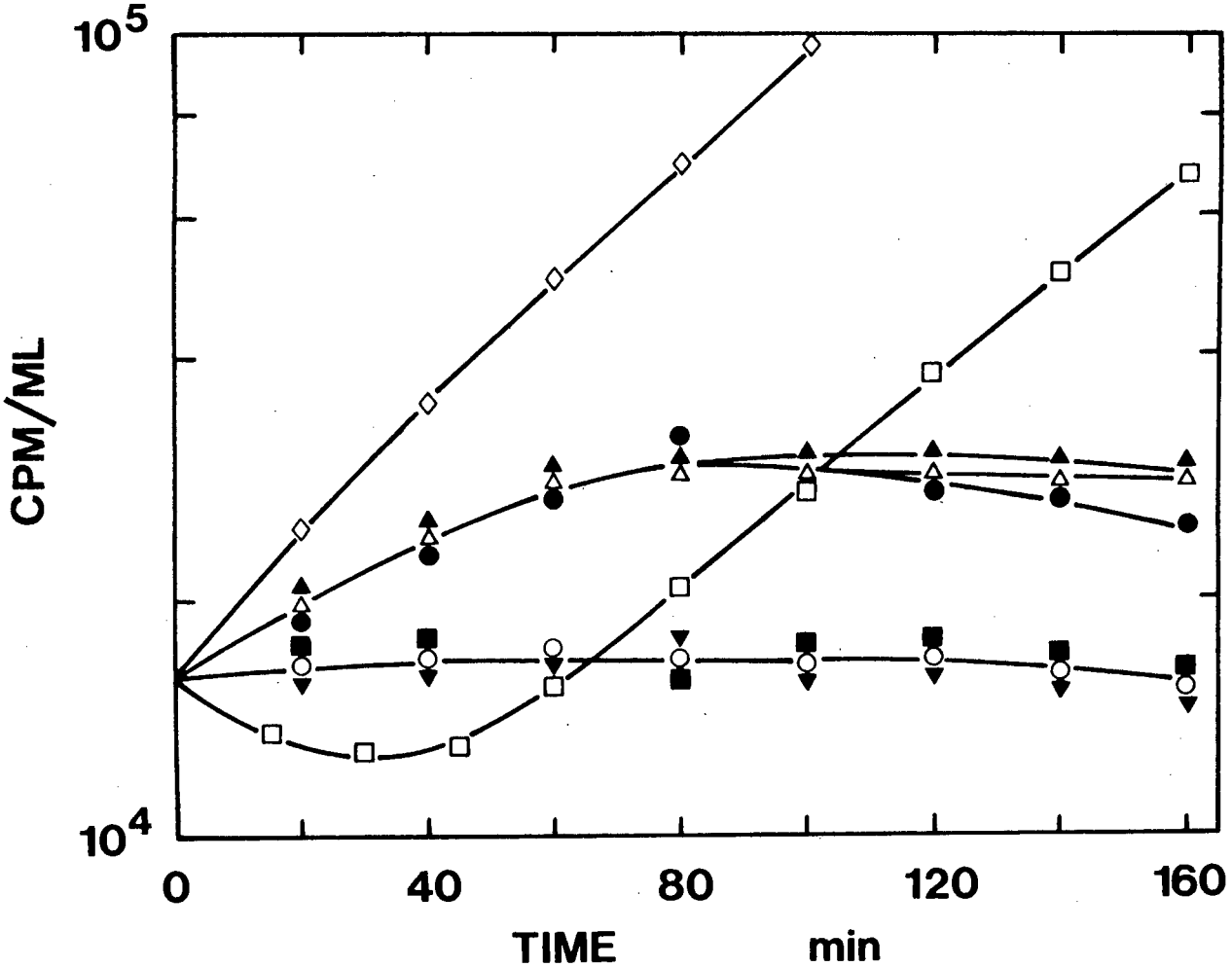

FIG 4.7 Effect of oxygen and UV radiation on DNA synthesis in prelabelled *B.fragilis* cells. The cells were labelled with [^{14}C] thymidine 60 min before exposure to oxygen and UV radiation. Unirradiated anaerobic control (◇). Cells irradiated (1,8% survival) and held under anaerobic conditions (□). Unirradiated cells exposed to oxygen in the absence (▲) and presence of either caffeine (1 mg ml^{-1}) (●) or chloramphenicol (5 µg ml^{-1}) (△). Irradiated cells (1,3% survival) exposed to oxygen in the absence (■) and presence of either caffeine (○) or chloramphenicol (▼).

4.3.4 Effect of UV radiation and oxygen on RNA and protein synthesis

Experiments with prelabelled cells irradiated under aerobic conditions indicated that RNA and protein synthesis were less sensitive to the effects of UV radiation and oxygen than DNA synthesis (Figs 4.4 and 4.5). RNA and protein synthesis continued after a UV dose which completely inhibited DNA synthesis (1% survival)(Figs 4.4, 4.5 and 4.6). RNA synthesis was inhibited after 80 min but protein synthesis continued for at least 160 min.

4.3.5 Effect of hydrogen peroxide on DNA synthesis and colony formation

The effect of hydrogen peroxide (0,0001 to 0,1%) on DNA synthesis in prelabelled *B.fragilis* cells was determined with and without oxygen (Fig 4.8a and 4.8b). Hydrogen peroxide concentrations between 0,0001 and 0,0025% did not affect DNA synthesis over a 60 min period after the addition of hydrogen peroxide under anaerobic conditions (Fig 4.8a). These concentrations of hydrogen peroxide reduced or inhibited DNA synthesis 60 to 80 min after the addition of hydrogen peroxide.

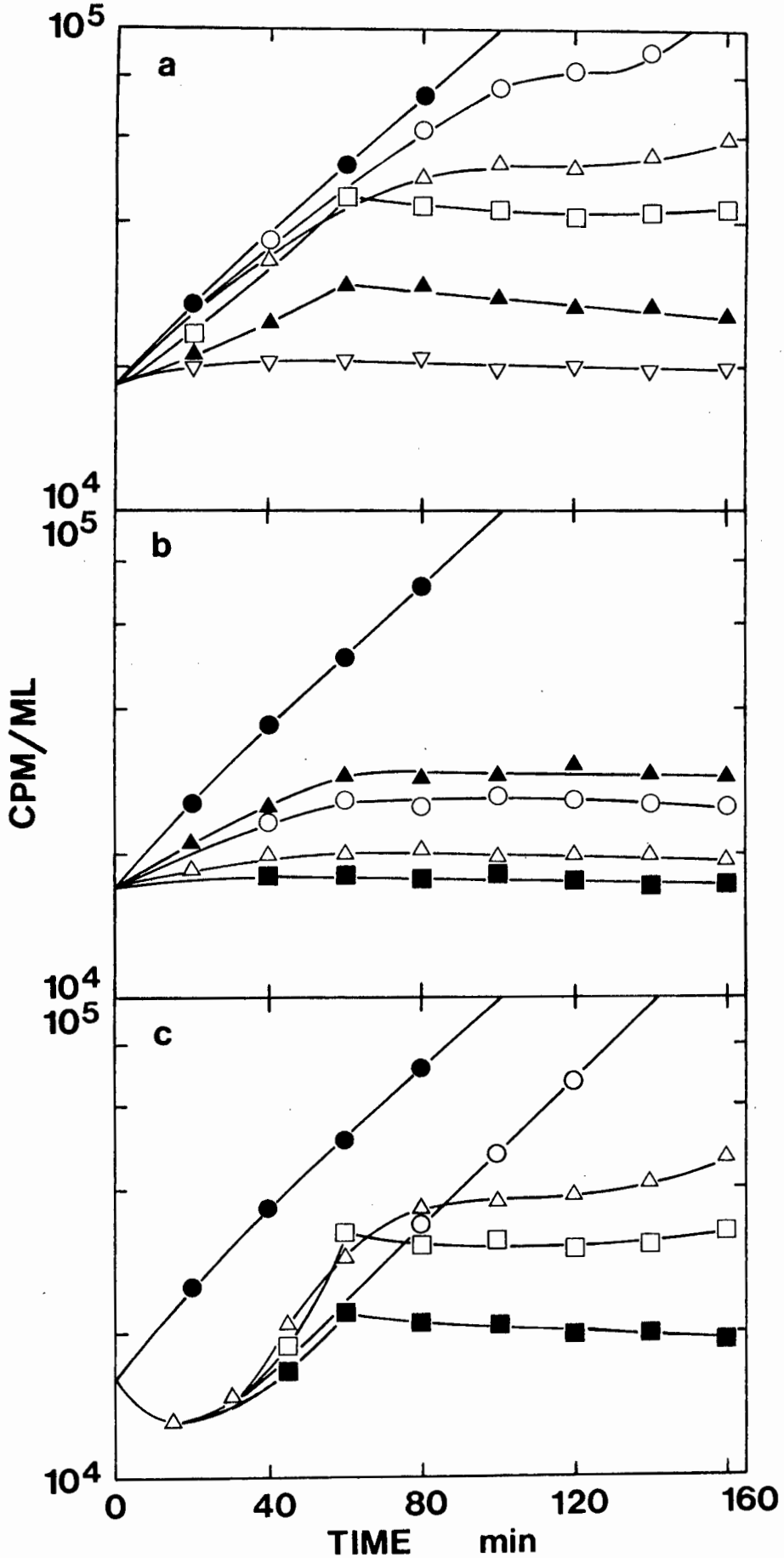
a
b
c
10^5
10^4
CPM/ML
0
40
80
120
160
TIME min

FIG 4.8. Effect of hydrogen peroxide, oxygen and UV radiation on DNA synthesis in prelabelled *B.fragilis* cells. The cells were labelled with [^{14}C] thymidine 60 min before exposure to hydrogen peroxide, oxygen or UV radiation. Effect of hydrogen peroxide on DNA synthesis under anaerobic conditions (a). Unirradiated cells exposed to hydrogen peroxide (0,0%, ●; 0,0001%, ○; 0,001%, △; 0,0025%, □; 0,01%, ▲; 0,1%, ▽). Effect of hydrogen peroxide on DNA synthesis under aerobic conditions (b). Unirradiated cells exposed to hydrogen peroxide (0,0%, ▲; 0,0001%, ○; 0,001%, △; 0,01%, ■) under aerobic conditions. Unirradiated anaerobic control without hydrogen peroxide (●). Effect of hydrogen peroxide and UV irradiation on DNA synthesis under anaerobic conditions (c). Irradiated cells exposed to hydrogen peroxide (0,001%, △; 0,0025%, □; 0,01%, ■) under anaerobic conditions. Unirradiated (●) and irradiated (○) anaerobic control without hydrogen peroxide. Cells were irradiated with 50 J m^{-2} UV (10,8% survival).

DNA synthesis was decreased by 0,01% hydrogen peroxide and completely inhibited by 0,1% hydrogen peroxide.

There was a synergistic effect between oxygen and hydrogen peroxide on DNA synthesis (Fig 4.8b) and concentrations of hydrogen peroxide (0,001%) which had no effect on DNA synthesis between 0 and 60 min under anaerobic conditions, inhibited DNA synthesis immediately in the presence of oxygen.

The effects of hydrogen peroxide and UV radiation were determined in prelabelled cells which were irradiated with 50 J m^{-2} UV light (10,8% survival) (Fig 4.8c). UV irradiation of cells prelabelled with [^{14}C] thymidine and exposed to between 0,001 and 0,01% hydrogen peroxide resulted in the initial rapid net loss of label from the trichloroacetic acid-precipitable fraction. The DNA degradation phase continued for approximately 40 to 60 min before net synthesis of DNA was observed at a similar exponential rate as in unirradiated cells. This synthesis continued for a period of time which was dependent on the concentration of hydrogen peroxide, before it was reduced or inhibited.

The effect of hydrogen peroxide on colony formation

in exponential *B.fragilis* cultures in minimal medium was determined under anaerobic conditions (Fig 4.1). Hydrogen peroxide (0,001%) did not markedly affect the viability of *B.fragilis* cells and only a slight decrease in rate of colony formation was observed as compared with the anaerobic control. In the presence of 0,01% hydrogen peroxide, colony formation continued at a reduced rate and was then inhibited between 60 to 90 min after addition of the hydrogen peroxide.

4.4 DISCUSSION

Although *B.fragilis* is an obligate anaerobe, it was able to survive and continue synthesizing DNA, RNA and protein for a limited period after exposure to oxygen. The rate and time of inhibition of macromolecular synthesis by oxygen was affected by the method of aeration, but it was always constant for a specific method. Our results on the effect of oxygen on molecular synthesis in *B.fragilis* are in marked contrast to the results of Glass *et al* (1979) with *Bacteroides thetaiotaomicron* and by Stevenson (1979) with *Bacteroides ruminicola*. In *B.thetaiotaomicron* the incorporation of radiolabelled thymidine and methionine into macromolecules is inhibited immediately and completely upon exposure to air, and direct chemical analysis of

macromolecules in this organism confirmed that oxygen in fact inhibits the synthesis of proteins and nucleic acids. Stevenson (1979) reported that in *B.ruminicola* exposure of the cells to air rapidly inhibits amino acid uptake. The reason for the difference between the *B.fragilis* Bf-2 strain and the other two *Bacteroides* species is not known but it suggests that there are substantial differences between species of *Bacteroides* and their response to oxygen. The *B.fragilis* Bf-2 strain was first isolated in 1977 and has been regularly exposed to air over the last 6 years which may account for its response to oxygen.

The plateau in radiolabelled thymidine incorporation in *B.fragilis* cells exposed to oxygen was not due to a shutoff of isotope transport, as was reported for aerated *B.ruminicola* cells. Active uptake of [^{14}C] thymidine in *B.fragilis* was shown to continue in cells exposed to oxygen after incorporation of label into DNA had been inhibited. DNA turnover experiments indicated that the plateau in fact reflected an overall net inhibition of DNA synthesis.

O'Brien & Morris (1971) reported that exposure of *C.acetobutylicum* cells to oxygen causes a bacteriostatic inhibition of DNA, RNA and protein synthesis

and that synthesis of these macromolecules resumes at exponential rates when the aerated cells are restored to anaerobic conditions. DNA synthesis in *B.fragilis* resumed at an exponential anaerobic rate after an initial lag period only if the aerated cells were restored to anaerobic conditions before the plateau in DNA synthesis was reached. Once the DNA synthesis was inhibited, no recovery of synthesis was observed during a further 1,5 hours of anaerobic incubation.

There was an interesting correlation between the inhibition of DNA synthesis by oxygen and loss of viability in *B.fragilis* cells. Within 10 to 20 min after inhibition of DNA synthesis a decrease in viability of *B.fragilis* cells occurred. The same correlation did not always hold true for *B.fragilis* cells which had been treated with hydrogen peroxide under anaerobic conditions. DNA synthesis in cells treated with 0,01% hydrogen peroxide was severely repressed and then inhibited, but although colony formation was inhibited, no significant decrease in viability was observed during an additional 80 min of incubation in the presence of hydrogen peroxide. Exposure of cells to oxygen differs from hydrogen peroxide treatment of cells in that oxygen exposure in addition may produce fluxes of superoxide radicals by cellular metabolism and photochemical oxidation

in the environment. Both Halliwell (1982) and Cancelliere *et al* (1975) reported that superoxide radicals can seriously damage cell membranes, and that there was a correlation between this structural damage and a loss in viability of *E.coli* K12 cells. Oxygen can also indirectly inhibit energy-producing biosynthetic pathways in anaerobes, since the primary electron donor NADH in cells preferentially binds to molecular oxygen, if present, but anaerobes presumably have very little scope for diverting the electron flow for the detoxification of oxygen without the inhibition of other NADH-mediated cellular processes (O'Brien & Morris, 1971; Morris, 1976). In the light of the discrepancies between viability and DNA synthesis in oxygen- and hydrogen peroxide-treated *B.fragilis* cells, as well as the oxygen-specific damaging effects in cells, it is not unlikely that cell death in the anaerobe *B. fragilis* after prolonged exposure to oxygen, results from several biochemical and physiological responses which become irreversible. In *E.coli* it was found that lethally damaged cells cease to replicate their DNA following a defined time-point of post-damaging treatment incubation (Trgovčević *et al*, 1981).

UV radiation in the presence of oxygen markedly affected DNA synthesis in *B.fragilis*, while RNA and protein synthesis were relatively unaffected.

The pattern of DNA synthesis in aerobically irradiated cells differed considerably from that in *B.fragilis* cells irradiated anaerobically in the presence or absence of hydrogen peroxide. Previously we reported that DNA synthesis in *B.fragilis* cells after UV irradiation under anaerobic conditions was characterized by an extensive degradation phase which continued for 40 min before net DNA synthesis was observed (Chapter 2). A similar pattern of DNA degradation followed by net DNA synthesis was observed after irradiation of hydrogen peroxide-treated cells. DNA synthesis in irradiated cells exposed to oxygen was immediately inhibited and no degradation of DNA was observed. *B.fragilis* differs from other bacteria in that molecular oxygen sensitizes the cells to far-UV radiation (Jones *et al*, 1980), but it was found that hydrogen peroxide does not have this sensitization effect (Slade *et al*, 1983b). Previously we reported that caffeine decreases the survival of *B. fragilis* cells irradiated under anaerobic conditions. (Jones *et al*, 1980; Chapter 3) and at the same time inhibits DNA degradation and DNA synthesis in irradiated cells (Chapter 2). It was thus suggested that the inhibition of DNA degradation and DNA synthesis by oxygen and UV radiation could be a reason for *B.fragilis* cells being more sensitive to UV irradiation in the presence of oxygen.

These studies on the effect of oxygen and hydrogen peroxide on macromolecular synthesis in unirradiated and UV-irradiated *B.fragilis* cells do not supply conclusive answers to the problems of oxygen toxicity in anaerobes or the oxygen sensitization of *B.fragilis* cells to far-UV irradiation. It does, however, characterize the macromolecular synthesis profiles in *B.fragilis* cells exposed to all the different DNA damaging treatments and as such provides a basis for further work on the possible relationship between the observed physiological responses of the anaerobe *B.fragilis* and DNA repair processes in the presence of oxygen.

CHAPTER 5

INDUCTION OF PROTEINS BY OXYGEN AND HYDROGEN PEROXIDE IN UNIRRADIATED AND UV-IRRADIATED *BACTEROIDES FRAGILIS* CELLS

5.1 INTRODUCTION

Oxygen cytotoxicity is held in check by a delicate balance between rates of generation of reduced oxygen species and the rate of their removal by different protective systems in the cell (Fridovich, 1978). Any shift in this balance may lead to cellular damage and living organisms have thus evolved several different defence mechanisms to protect themselves against the deleterious reduced oxygen species (Fridovich, 1975 and 1978). Some of these defence mechanisms, like the oxygen free radical scavenging enzymes NADH oxidase, catalase and superoxide dismutase (Morris, 1975; Hassan & Fridovich, 1977a) are induced in cells by the presence of their substrates.

Since anaerobes may at some time encounter oxygen, their response to this strong oxidant and its free radicals is of great interest. Contrary to earlier beliefs, it has been shown that in many anaerobes protective enzymes are present, and even inducible by exposure to oxygen, presumably to enable anaerobes to survive transient exposures to oxygen. A basic minimum level of the protective enzymes is present in most

anaerobes in their uninduced anaerobic state: the complete repression of the enzymes in the absence of their substrate would lead to the death of the anaerobe if it is suddenly exposed to an aerobic environment.

The scavenging ability of optimum levels of the protective enzymes in anaerobes may be exceeded by continuous and excessive aeration (Morris, 1975). All of the oxygen free radicals are capable of attacking and damaging DNA (Chapter 4) and Fridovich (1974) speculated that some irreversible oxygen free radical-mediated damage occurs in all organisms in spite of the existence of the protective enzymes. Unrepaired DNA damage can become a lethal event and it is therefore not unreasonable to anticipate that excessive oxidative DNA damage may elicit induction of repair proteins in living cells. Although obligate anaerobes exhibit an adverse sensitivity to oxygen (Morris, 1975 and 1976) and *B.fragilis* cells are also more sensitive to far-UV irradiation under aerobic than under anaerobic conditions (Jones *et al*, 1980), a comparison of the proteins induced in anaerobes by oxygen and hydrogen peroxide, and by far-UV irradiation under anaerobic and aerobic conditions, has not been reported.

5.1.1 Oxygen reduction and NAD(P)H oxidases

One of the primary defences of organisms against oxygen toxicity is the diversion of intracellular reducing power to preferentially reduce molecular oxygen. The oxygen reacts intracellularly mainly with NADH drawn from the intermediate metabolism of the cells. It has been shown that oxygen does not react at a significant rate with NADH in free solution, but enzyme-bound NADH is very reactive towards oxygen (Bielski & Chan, 1976). O'Brien & Morris (1971) were the first investigators to observe that the increase in the whole cell potential oxygen consumption (respiratory rate) which occurs when certain anaerobes are exposed to oxygen, coincides with the induction of the oxygen-reducing enzyme NAD(P)H oxidase, in these organisms. They reported that the anaerobe *C.acetobutylicum* adapts to oxygen exposure by increasing its NADH oxidase content, and since then similar systems have been reported for *Selenomonas ruminantium* (Wimpenny & Samah, 1978), *Clostridium sporogenes* and *Clostridium bifermentans* (Ashley & Shoesmith, 1977). Very little is known about NADH oxidases and peroxidases and the protection of anaerobes by these enzymes (Hoshino *et al*, 1978), but Wimpenny & Samah (1978) found it interesting that the induction pattern of NADH oxidase in the Gram-negative *S.ruminantium* was the same as in the Gram-positive

C.acetobutylicum . They described the NADH oxidase *S.ruminantium* as a soluble enzyme which most probably is a flavoprotein.

NAD(P)H oxidase is generally known as a hydrogen peroxide producing enzyme (Anders *et al*, 1970; Low & Zimkus, 1973). In some organisms this hydrogen peroxide can be converted to water by a NADH peroxidase (Mizushima & Kitahara, 1962; Dolin, 1977). Other organisms possess a NADH oxidase which directly produces water from oxygen without the intermediate production of hydrogen peroxide. These organisms would have a definite survival advantage over the former group, and this latter type of NADH oxidase has been described for *Clostridium perfringens* (Dolin, 1959), *Streptococcus faecalis* (Hoskins *et al*, 1962) and *Leuconostoc mesenteroides* (Kawai *et al*, 1971). The anaerobe *Peptostreptococcus anaerobius* possesses both a NADPH oxidase which forms hydrogen peroxide as an intermediate oxygen radical and a NADH oxidase which converts oxygen directly to water (Hoshino *et al*, 1978). This anaerobe can survive considerable exposure to oxygen and it was found that oxygen reacts intracellularly mainly with the NADH oxidase to produce water and the deleterious hydrogen peroxide is only formed to a limited extent.

Increasing the oxygen concentration above a certain critical level causes a rapid drop in NADH oxidase

activity and respiratory rates in anaerobic bacteria. This coincides with a growth inhibition which presumably happens because the rate of consumption of NADH via NADH oxidase exceeds the rate at which it could be supplied for this purpose without hazarding its other essential energy-producing functions in growing cells (O'Brien & Morris, 1971).

Lipsky & Hylemon (1980) reported a molecular weight of 260 000 for the NADH flavin oxyreductase from the anaerobe *Eubacterium lentum* while NADH flavin oxidases from other bacteria have molecular weights between 23 000 and 30 000, and a molecular weight of 43 000 was reported for the only NADPH oxidase isolated (Jablonski & DeLuca, 1977; Hasan & Nester, 1978; Lipsky & Hylemon, 1980).

5.1.2 Catalases

The oxygen free radical, hydrogen peroxide, has repeatedly been shown to have a highly deleterious effect on the integrity of DNA (Ananthaswamy & Eisenstark, 1977; Hartman & Eisenstark, 1978; Carlsson & Carpenter, 1980). In addition, it has been found that this radical is very stable in solutions and can thus accumulate appreciably in aqueous media. Awareness of this threat to living organisms led to the formulation of the catalase theory.

According to this theory the possession of the hydrogen peroxide scavenging enzymes catalase and/or peroxidase (Saunders,1973; Stadtman, 1980) is essential for survival of living organisms in air, and the lack of these enzymes in anaerobes accounts for their oxygen sensitivity. Holdeman & Moore (1972) showed, however, that a large number of obligate anaerobes produce these enzymes and Hanson & Stewart (1978) recently recommended a catalase test as a rapid screening procedure to identify members of the *"Bacteroides fragilis"* group of anaerobic Gram-negative bacilli.

Bacteroides distasonis is the anaerobe which produces the most catalase: the catalase levels reported for this organism are comparable with the level observed in the aerobic *E.coli* (Gregory & Fridovich, 1973a) and aero-tolerant *S.faecalis* organisms (Gregory & Fridovich, 1973b). The *B.distasonis* catalase enzyme has a molecular weight of 250 000 and its uninduced production reaches peak values in late logarithmic phase (Gregory *et al*, 1977a).

Gregory & Fridovich (1973b) claimed that catalase is not induced by oxygen in *E.coli* cells, but Hassan & Fridovich (1977a) reported a 2,4-fold increase in catalase and a 12,7-fold increase in peroxidase levels upon aeration of *E.coli* K12 cells. The induction of catalase by oxygen in anaerobes is markedly influenced by the growth medium.

Catalase is a non-dialyzable, cyanide- and azide-sensitive, heat-labile protein (Gregory *et al*, 1977a) which, with a few exceptions (Johnston & Delwiche, 1965) contains heme as a biologically active centre component (Bomberg & Luse, 1963; Schonbaum & Chance, 1976). *B.fragilis* cannot synthesize its own heme and must therefore transport preformed hemin for synthesis of heme proteins like catalases and cytochromes (Sperry *et al*, 1977). Several other *Bacteroides* species also require preformed hemin for catalase production, and catalase levels of *B.distasonis* were found to vary with the amount of hemin supplied to the medium (Gregory *et al*, 1977a). Ten times as much hemin is required for catalase production than for optimum growth (Wilkins *et al*, 1978). Since it is heme availability and not total heme concentration which is the important factor in determining catalase production, higher levels of catalase can be induced in some media if the hemin is added after autoclaving. The effect of hemin on catalase production is very specific and cannot be duplicated by ferrous sulphate or ferrous ammonium citrate (Gregory *et al*, 1977a). Vitamin K apparently acts synergistically with hemin in elevating the catalase specific activity (Gregory *et al*, 1977a). The requirement for exogenously supplied hemin was not recognized until recently and is one of the reasons why catalase production in anaerobes remained undetected for such a long time.

Gregory *et al* (1977b) showed that catalase production and induction in a number of *Bacteroides* species is suppressed by the presence of glucose and other carbohydrates in the medium. When cells grown in the absence of carbohydrates are transferred to media containing glucose, the residual catalase activity already in the cells is not destroyed, but is diluted out as the cells multiply without producing more enzyme. *B.fragilis* produces relatively large amounts of catalase (25 to 50 units mg^{-1} of protein) in the absence of carbohydrates (Gregory *et al*, 1977b). Anaerobes are routinely cultured in media that contain carbohydrates as energy sources and this may be another reason why catalase production was not previously detected in anaerobes.

Several investigators have stated that catalase induction does not render cells more tolerant towards oxygen (Gregory & Fridovich, 1973a and 1973b; Fridovich, 1974; Rolfe *et al*, 1978) or less sensitive to hydrogen peroxide (Adler, 1963; Ananthaswamy & Eisenstark, 1977). Although catalase significantly reduces the concentration of hydrogen peroxide present in cells during aerobic metabolism (Schonbaum & Chance, 1976) its *in vivo* activity is affected by several environmental conditions. The endogenous catalase in thermally stressed *Staphylococcus aureus* cells is inactivated by the combined action of heat and the NaCl in selective *Staphylococcus* media; exogenously added catalase was found to cause as much

as a 15 000-fold increase in the enumeration of these thermally stressed *S.aureus* cells (Martin *et al*, 1976). Catalase is also inactivated both by high concentrations of hydrogen peroxide, and by cysteine, which is a standard component of anaerobic media (Boeri & Bonnichsen, 1952; Alexander, 1957). Peroxidase enzymes which are effective scavengers of hydrogen peroxide and are capable of using cysteine as a substrate in the reaction (Olsen & Davis, 1976), are therefore much better suited to protect anaerobes (e.g. *P.anaerobius*) against the bactericidal effect of aerated cysteine-containing media (Carlsson *et al*, 1979).

5.1.3 Superoxide dismutases

The cytotoxicity of the superoxide radical is well established (Halliwell, 1982) and it is thus important for all bacteria to dispose of these highly damaging superoxide radicals in a relatively innocuous way. McCord & Fridovich (1969) were the first to report the existence in bacteria of superoxide dismutase enzymes (SODs). SOD very efficiently catalyzes the safe dismutation of superoxide radicals to yield hydrogen peroxide and triplet oxygen (Morris, 1976). SOD can also effectively quench singlet oxygen (Fridovich, 1974) and in *E.coli* cells a definite correlation seems to exist between oxygen sensitivity and a lack of SOD

production in mutants of this organism (Hassan & Fridovich, 1979).

In 1971 McCord *et al* proposed the superoxide dismutase theory of obligate anaerobiosis in which they stated that the lack of this important enzyme accounted for the aero-intolerance of anaerobes. The production of this enzyme is, however, not restricted to aerobic and aerotolerant bacteria and a few years after McCord *et al* proposed their theory, SOD was discovered in several aero-intolerant, and even strict anaerobic, bacteria (Hewitt & Morris, 1975; Ashley & Shoesmith, 1977; Gregory *et al*, 1978). The aero-intolerant bacteria in which the presence of SOD has been demonstrated include *Chlorobium*, *Desulfovibrio*, *Clostridium* (Morris, 1976) and several *Bacteroides* species (Carlsson *et al*, 1977; Gregory *et al*, 1977a; Tally *et al*, 1977; Rolfe *et al*, 1978), and in the anaerobes *Bacteroides* (Carlsson *et al* 1977; Tally *et al*, 1977), *Clostridium* (Ashley & Shoesmith, 1977) and *S. ruminantium* (Wimpenny & Samah, 1978; Samah & Wimpenny, 1982) the levels of SOD present in the cells correlate well with the oxygen tolerance of these organisms. The SOD level in certain *Bacteroides* species is similar to or even greater than that reported for *E.coli* (Gregory *et al*, 1978). The presence of SOD in strict anaerobes has been explained in different ways. The most plausible explanation is that the presence of SOD enables them to survive

transient exposures to oxygen, albeit with growth inhibition (Tally *et al*, 1977; Halliwell, 1982). Lumsden & Hall (1975) thought that SOD in anaerobes provides protection against superoxide radicals formed by cosmic radiation, while Tally *et al* (1977) also proposed that SOD is used as a virulence factor by pathogenic anaerobes.

There are four different types of SODs known. Copper- and zinc-containing superoxide dismutases (Cu/Zn SOD) are characteristic of the cytosol of eucaryotic cells, although similar enzymes have been found in the procaryotes *Photobacterium leioyhathi* and *Micrococcus denitrificans* (Bannister & Bannister, 1981). Manganese SODs (MnSODs) are common to both procaryotes (Vance *et al*, 1972; Weisiger & Fridovich, 1973) and eucaryotes (Bannister & Bannister, 1981), but SODs containing iron (FeSODs) have only been isolated from procaryotes (Bannister & Bannister, 1981). In *E.coli* a MnSOD is found in the cell matrix (Keele *et al*, 1970; Gregory *et al*, 1973) and presumably protects the organism against endogenously produced superoxide radicals (Rolfe *et al*, 1978), while FeSOD is found in the periplasmic space (Gregory *et al*, 1973; Yost & Fridovich, 1973) and may protect against exogenously generated superoxide radicals. This latter type of protection may be important for anaerobes which reduce little or no oxygen internally and will also be advantageous in the establishment of infections in well

oxygenated tissues (Rolfe *et al*, 1978).

The MnSOD in *E.coli* cells is inducible by exposure to oxygen, while the FeSOD is constitutively produced in this organism (Hassan & Fridovich, 1977a). A second, previously undescribed SOD is also induced in anaerobically grown *E.coli* K12 cells exposed to air (Hassan & Fridovich, 1977a). The induction of SOD is rapid (Fridovich, 1974), but the two inducible SODs in *E.coli* differ in their responsiveness to oxygen: very low levels of oxygen cause a near maximal induction of MnSOD, while the induction of the new SOD requires much higher levels of oxygen. Consequently Hassan & Fridovich (1977a) suggested that the differences in oxygen sensitivity between *E.coli* K12 and *E.coli* B may be quantitative rather than qualitative. The non-inducibility of FeSOD may be due to a deficiency of iron as a result of the induction of cytochromes and other iron-containing proteins upon aeration of *E.coli* cells.

The SOD in *E.coli* can be induced to 25 times the anaerobic level (Gregory & Fridovich, 1973b), while the maximum level of SOD in *S.faecalis* is 16-fold higher than the uninduced level of SOD present in the cells during anaerobic growth (Gregory & Fridovich, 1973b). The increase in intracellular SOD correlates well with a gain in resistance to hyperbaric oxygen in *E.coli*, *S.faecalis*

and *Saccharomyces cerevisiae* (Fridovich, 1972; Gregory & Fridovich, 1973a and 1973b; Fridovich, 1974; Gregory & Fridovich, 1974; Fridovich, 1975; Hassan & Fridovich, 1977a). *Bacteroides* species contain a low level of SOD in cells grown under anaerobic conditions; when these cells are exposed to 2% oxygen, growth is inhibited and the SOD content increases to 5 times the anaerobic level (Gregory *et al*, 1977a).

Hassan & Fridovich found that intracellular concentrations of SOD in *E.coli* cells can be manipulated not only by aeration (1977a) but also by changing the metabolism from fermentative to respiratory (1977c). Glucose indirectly depresses the level of SOD in *E.coli*, since the fermentative catabolism of glucose is associated with a low rate of superoxide radical production (Hassan & Fridovich, 1977c). Once the glucose in the medium is exhausted, or the pH has fallen low enough because of an accumulation of organic acids, a shift from fermentative to predominantly oxidative catabolism occurs. The oxidative degradation of the nitrogenous compounds generates many more superoxide radicals, and the SOD levels in the organism are elevated under these conditions (Hassan & Fridovich, 1977c). A change in the growth rate of chemostat cultures also appears to affect the SOD levels in *E.coli* cells (Hassan & Fridovich, 1977b).

The presence of the SOD enzyme can be detected both in solutions and in gels. In solution SOD prevents the auto-oxidation of pyrogallol (1,2,3-benzenetriol)(Marklund & Marklund, 1974), while the presence of SOD can be detected in polyacrylamide gels as brown bands against an unstained background (Misra & Fridovich, 1977) or achromatic bands against a blue background (Beauchamp & Fridovich, 1971). The latter is the most often used detection system and is based on the inhibition by SOD of the superoxide radical-mediated reduction of nitroblue tetrazolium to blue formazan (Rajagopalan & Handler, 1964; Beauchamp & Fridovich, 1971; Morris, 1975).

Rolfe *et al* (1978) found three bands which showed SOD activity when aerobically grown *E.coli* cells were resolved on polyacrylamide gels, whereas anaerobically grown cells only exhibited two SOD bands. Halliwell (1982) mentioned that *E.coli* loses it MnSOD when grown anaerobically, but retains its FeSOD, presumably to allow it to survive re-exposure to oxygen (whereupon it promptly resynthesizes the MnSOD). Rolfe *et al* (1978) stated that most anaerobes contain at least two electrophoretically distinct SOD enzymes and that the Rf values of SODs from *B.fragilis* and *C.perfringens* are very similar. *Bacteroides vulgates* possesses three SOD enzymes, two with Rf values similar to the SODs in *B.fragilis*. *Eubacterium lentum* is the only anaerobe studied so far which exhibits a single band of SOD activity (Rolfe *et al*, 1978).

SOD is a metalloprotein which operates with extra-ordinary catalytic efficiency (Fridovich,1974). The catalytic action of SOD is due to alternate reduction and reoxidation of the active site metal during successive encounters with superoxide radicals (Bannister & Bannister, 1981). The apoenzyme is devoid of catalytic activity and activity is only regained once the metal prosthetic group is restored (Fridovich, 1981). The complete amino acid sequence has been determined for the MnSODs from *E.coli* (Steinman, 1978) and *Bacillus stearothermophilus* (Bannister & Bannister, 1981), but no FeSOD has been sequenced in full as yet. There exist extensive sequence homologies between N-terminals of FeSOD and MnSOD (Harris *et al*, 1980). FeSOD is a dimer, while MnSOD can be dimeric or tetrameric, and Bannister & Bannister (1981) reported that these enzymes are composed of identical, non-covalently bound subunits of molecular weight 22 000 to 23 000. Fee (1981) quoted the molecular weight of FeSOD to be 38 000 and the molecular weight of the inducible MnSOD as 40 000/80 000, while Gregory *et al* (1977a) reported the molecular weight of the SOD in *B. distasonis* to be 40 000.

5.1.4 Synergism amongst protective enzymes

It is highly selective to choose any particular protein

to account for an organism's resistance to oxygen damage in the face of the pleiotropic response of the cell to excessive aeration. A more likely explanation for the observed oxygen tolerance in organisms would entail the synergistic interaction between two or more of the protective enzymes.

One of the byproducts of oxygen reduction by NADH oxidase is the superoxide radical (Wimpenny & Samah, 1978). In the anaerobe *S. ruminantium* a SOD enzyme is induced at the same time as the NADH oxidase and complements the NADH oxidase action in protecting these organisms against oxygen toxicity (Wimpenny & Samah, 1978; Samah & Wimpenny, 1982). Wimpenny & Samah reported in 1978 that the inducible SOD responded to higher oxygen concentrations than the NADH oxidase and that SOD levels were highest in cells whose growth was completely inhibited by oxygen. In 1982, however, they modified this statement and reported that the activity of SOD declines at high oxygen concentrations. The induction of NADH oxidase reaches a maximum at a higher aeration level than the aeration level necessary to evoke maximum SOD production. Both NADH oxidase and SOD activities decrease at oxygen concentrations high enough to inhibit growth in *S. ruminantium* (Samah & Wimpenny, 1982).

The catalase enzyme is inhibited by fluxes of superoxide radicals: these radicals are small enough to gain access to the hemes of catalase and convert the resting enzyme to the inactive ferro-oxy state (Kono & Fridovich, 1982). This inhibition occurs in two distinct ways: the first type of inhibition is rapidly established and can be prevented and reversed by SOD, while the second type develops slowly and is reversed by ethanol but not by SOD. In any reaction mixture generating both hydrogen peroxide and superoxide radicals, the effective scavenging ability of catalase is therefore enhanced by the simultaneous presence of active SOD. In addition, superoxide radicals also react with peroxidases, rendering them relatively inactive (Odajima & Yamazaki, 1970 and 1972), and the presence of SOD in the reaction mixture also relieves this inactivation by harmlessly dismutating the superoxide radicals (Odajima & Yamazaki, 1972).

Hydrogen peroxide radicals, on the other hand, can inactivate Cu/Zn and FeSODs (Beauchamp & Fridovich, 1973; Asada *et al*, 1975; Hodgson & Fridovich, 1975) and catalase consequently markedly enhances the protective effect afforded to organisms by SOD (Kellogg & Fridovich, 1977). Kono & Fridovich (1982) thus concluded that catalase and SOD constitute a mutually protective set of enzymes.

5.1.5 Influence of growth media on the production of protective enzymes

The induction and production of both SOD and catalase in bacteria are influenced by the nature of the suspending medium. Rolfe *et al* (1978) found that anaerobic bacteria grown on the surface of agar plates produced more of the protective enzymes than bacteria grown in liquid media, while Wilkins *et al* (1978) reported that more catalase was produced by *Bacteroides* cells growing in broth than in the same type of media prepared with agar.

E.coli cells grown in complete media show higher levels of SOD when exposed to fluxes of superoxide radicals generated by paraquat under aerobic conditions, than those cells grown in minimal medium (Moody & Hassan, 1982). In complete media there is a linear correlation between SOD content and the concentration of paraquat, whereas in minimal medium the cells are unable to increase their level of SOD beyond that caused by a paraquat concentration of 0,1mM. Fluxes of superoxide radicals have no effect on the catalase level in cells in complete medium, but catalase is increased by 2- to 4-fold in cells grown in minimal medium and subsequently exposed to paraquat under aerobic conditions (Moody & Hassan, 1982). In general it therefore appears as if superoxide radicals are more toxic in a simple nutritionally restricted medium than in a rich complex medium.

5.1.6 Effect of oxygen at the genetic/metabolic level

The induction of SOD and catalase by oxygen has been seen by many investigators as a form of adaptation of living organisms to hyperoxia (Fridovich, 1981). Nierlich (1978) found that bacteriostatic hyperoxia actually evokes a stringency response. Stringency is a pleiotropic response under the control of the *rel* genes (Gallant, 1979) and subjects the whole of the cellular metabolism to major changes. The inability to exhibit a stringency response and thus to adapt to hyperoxia, leads to cell death in hyperoxygenated *relA*$^-$ *E.coli* cells (Fee, 1981). Chloramphenicol, tetracycline and rifampicin can interfere with the onset of stringency (Sokawa & Sokawa, 1978; Glass *et al*, 1979) and Fee (1981) suggested that the induction of MnSOD is part of this response.

The onset of stringency is characterized by the marked accumulation of the regulatory nucleotide guanosine 5'-diphosphate 3'-diphosphate (ppGpp) (called the "engine of stringency" by Fiil *et al*, 1977). The involvement of both ppGpp and guanosine 5'-triphosphate 3'-diphosphate (pppGpp) in the regulation of a number of gene functions and a variety of bacterial operons has been reported (Cashel & Gallant, 1974; Cashel, 1975; Stephens *et al*, 1975). This regulation occurs

at the RNA level in *E.coli* by the restriction of the transcription of rRNA by ppGpp (Arbige & Chesbro, 1982).

However, stringency is not a result of oxygen poisoning alone. Arbige & Chesbro (1982) found that a falling glucose level evokes ppGpp accumulation in *E.coli* cells grown in a continuous fermenter, and several other investigators reported that ppGpp accumulates in bacteria after any environmental perturbation affecting the energy supply system in the cells (Lazzarini *et al*,1971; Lund & Kjeldgaard, 1972; Hansen *et al*, 1975; Johnsen *et al*, 1977; Jacobson & Jacobson, 1980; Richter, 1980). Glass *et al* (1979) made the interesting discovery that aeration of the obligate anaerobe *Bacteroides thetaiotaomicron* leads to the marked increase in the concentrations of both ppGpp and pppGpp. This was the first report of the phenomenon of ppGpp and pppGpp accumulation in anaerobes. Glucose exhaustion in the growth medium can elicit the same response in *B.thetaiotaomicron* and Glass *et al* (1979) postulated that the impairment of glycolysis by oxygen serves to deplete the pool size of a key metabolite(s). This could then trigger the induction of a typical stringency reponse in *B. thetaiotaomicron*, which becomes evident in the abrupt cessation of macromolecular synthesis in cells exposed to air (Glass *et al*,1979).

A bacteriostatic inhibition of growth is part of the stringency reponse in *B.thetaiotaomicron* (Glass *et al*, 1979). In *E.coli* it was found that the synthesis of membranes and septa and the elongation of cells are also regulated by ppGpp (Nierlich, 1978; Gallant, 1979). At concentrations of ppGpp not high enough to affect RNA polymerase and transcription of rRNA, the activity of the enzymes involved in phospholipid and peptidoglycan synthesis in *E.coli* is affected and growth becomes restricted (Arbige & Chesbro, 1982). "Primed sites" accumulate in areas of cell wall addition and incipient septation (Sloan & Urban, 1976) and cells are poised to complete cell elongation, septum formation and cell division. This potential is realized in *E.coli* upon restoration of normal energy levels (Arbige & Chesbro, 1982). The available energy enables the product of the *spoT* gene, guanosine 3',5'-bis (diphosphate) 3'-pyrophosphohydrolase (ppGppase) to become functional again and to rapidly degrade ppGpp (De Boer *et al*, 1975; Tetu *et al*, 1980). It is not clear whether an analogous mechanism of derepression may be responsible for the resumption of growth and macromolecular synthesis in anaerobes such as *C.acetobutylicum* where the restoration of this organism to anaerobic conditions coincides with a rise in intracellular ATP levels (O'Brien & Morris, 1971).

Although the induction in anaerobes of the protective

enzymes, NADH oxidase, catalase and SOD, by oxygen has been extensively studied, nothing is known about the induction of DNA repair proteins under aerobic conditions in anaerobic bacteria. In the anaerobe *B.fragilis* phage reactivation is induced by both oxygen and hydrogen peroxide (Slade *et al*,1983a and 1983b). However, the presence of oxygen increases the sensitivity of *B.fragilis* cells to far-UV irradiation (Jones *et al*,1980), whereas pretreatment of the cells with hydrogen peroxide increases the survival of both irradiated phage and cells (Slade *et al*, 1983b). Oxygen does not affect the number of pyrimidine dimers induced by far-UV irradiation in *B.fragilis*, but LHR in this organism only occurs under aerobic conditions (Jones & Woods, 1981); LHR in *E.coli* is only observed in *recA* mutants (Ganesan & Smith, 1968). It has been shown that although the presence of oxygen sensitizes *E.coli* and *Closterium moniliferum* cells to killing by X-rays, oxygen does not influence the induction of X-radiation resistance (i.e. repair proteins)(Bryant, 1976; Howard & Cowie, 1978).

In order to elucidate the relationship between the cell survival characteristics and phage reactivation systems induced by oxygen, hydrogen peroxide and UV light in the anaerobe *B.fragilis*, we compared the number and molecular weights of the proteins induced by these DNA-damaging agents in this organism.

5.2 MATERIALS AND METHODS

5.2.1 Bacteria and media

These studies were carried out on a *B.fragilis* strain (Bf-2) which has been described in section 2.2.1 and was used in our previous studies on far-UV irradiation (Jones *et al*, 1980; Jones & Woods, 1981; Slade *et al*, 1981, 1983a and 1983b). Brain heart infusion broth and agar, supplemented with hemin, menadione, and cysteine (Holdeman & Moore, 1972) were used for bacterial propagation at 37°C. Prereduced one-quarter-strength Ringer solution was used as a dilution buffer (Jones & Woods, 1981). Irradiation and radioactive labelling of the cells were carried out in a defined minimal medium (Varel & Bryant, 1974).

5.2.2 Anaerobic and aerobic treatments

All manipulations and treatments requiring anaerobic conditions were carried out in an anaerobic glove cabinet (Forma Scientific, Marietta, Ohio) in an atmosphere of 70% N_2, 20% CO_2 and 10% H_2. Cultures were aerated in an aerobic incubator by shaking 4ml samples in 14ml glass centrifuge tubes. The redox indicator, resazurin, in the medium was pink during the aerobic treatments but was colourless under anaerobic conditions.

5.2.3 Induction by oxygen and hydrogen peroxide and labelling of proteins

Overnight cultures of *B.fragilis* in brain infusion broth were diluted 100-fold in minimal medium and reincubated until the cultures reached a turbidity of 0,2 at 600nm (1×10^8 to 2×10^8 c.f.u. ml^{-1}) and were in early exponential phase. Samples (4 ml) were with exposed to oxygen or treated with sublethal concentration of hydrogen peroxide (0,0025%) for different time intervals (0, 10 and 25 min). Both these treatments have been shown to induce phage reactivation (Slade *et al*, 1983a and 1983b). Samples (2 ml) of the induced cells were pulse-labelled for 10 min at 37°C by the addition [^{35}S] methionine (Radiochemical Centre, Amersham, England)(10 µg ml^{-1}; 40 µCi ml^{-1}). After labelling in the presence of oxygen and/or hydrogen peroxide the cells were collected by centrifugation with a microfuge, washed twice with Ringer solution, and suspended at a 40-fold concentration of the original cell volume in electrophoresis buffer containing sodium dodecyl sulphate (SDS). The samples were then boiled for 2 min and either resolved immediately by polyacrylamide slab gel electrophoresis (PAGE) or stored at -20°C until required.

5.2.4 UV irradiation

The effect of UV radiation on oxygen- and hydrogen-

peroxide treated cells and the induction of protein was determined. Samples (9 ml) of exponential phase cultures were irradiated in open glass petri dishes with a Fluotest Piccolo Hanau Quartz germicidal lamp (254 nm). The dose rate was measured with a Blak-Ray UV meter (model J-225; UV Products Inc., San Gabriel, Calif.) and samples were irradiated at a dose rate of 1 J $m^{-2}s^{-1}$. In experiments where the effect of UV and oxygen on the induction of proteins was studied, the exponential phase culture were exposed to air for a few seconds before irradiation in the presence of oxygen. Irradiation was not carried out in the presence of hydrogen peroxide, but the hydrogen peroxide was added to the cultures immediately after UV irradiation. Survival curves for cells irradiated with increasing UV influences were determined and labelling experiments were routinely carried out with cultures irradiated to a survival level of approximately 0,1%.

5.2.5. Effect of caffeine

The effect of caffeine on the production of oxygen-induced proteins was determined. The minimal inhibitory concentration of caffeine was 2,5 mg ml^{-1}. Caffeine (1 mg ml^{-1}) was added to the broth cultures immediately and 25 min after exposure to oxygen. When caffeine was added immediately after exposure to oxygen, the cells

were held in the presence of caffeine for 25 min before labelling with [^{35}S] methionine. Cells that were exposed to oxygen for 25 min before caffeine was added, were held for 10 min in the presence of the inhibitor before labelling with [^{35}S] methionine.

5.2.6 Electrophoresis and autoradiography

The labelled proteins were subjected to discontinuous SDS-8,4% PAGE according to the methods described by Laemmli (1970) and O'Farrell (1975). The samples (20 µl) were stacked at 100V and then resolved on 0,5 mm-thick Hoefer slab gels at 30 mA per gel at 4°C. The gels were stained with Coomassie brilliant blue (0,05%, wt/vol), destained, washed and dried. Labelled protein bands were visualized by exposing the dried gels to Kodak X-Omat MA X-ray film at -20°C for 28 to 42 days (Lasky & Mills, 1975). Molecular weight markers were supplied by BDH Biochemicals Ltd. (Poole, England) and consisted of cross-linked polymers of a purified degradation product of myoglobin with a molecular weight range from 14 300 to 71 500. Purified human transferrin (molecular weight 90 000; Sigma Chemical Co., St. Louis, Mo.) was used as an additional marker. The autoradiograph strips were scanned with a Beckman DU-8 spectrophotometer with a gel scanner attachment.

5.3 RESULTS

5.3.1 Oxygen induction of proteins

In eight independent experiments exposure of exponential phase *B.fragilis* cells to oxygen resulted in the induction of a new 37 000-molecular weight protein (protein 5) and the induced synthesis of three other proteins with molecular weights of 106 000, 90 000 and 70 000 (proteins 6, 2 and 3, respectively)(Figs 5.1 and 5.2). The 106 000-, 90 000- and 70 000-molecular weight proteins were synthesized in small amounts in anaerobic cells and the induced synthesis of the 90 000- and 70 000-molecular weight proteins (proteins 2 and 3) was observed previously in cells irradiated with UV light under anaerobic conditions (Chapter 3; Schumann *et al*, 1982). The production of a 40 000-molecular weight protein (protein 4) was inhibited by oxygen. The other labelled proteins in the autoradiograms were not affected by oxygen and showed relatively minor variations after exposure to oxygen (Figs 5.1 and 5.2).

5.3.2 Hydrogen peroxide induction of proteins

Treatment of *B.fragilis* cells with hydrogen peroxide under anaerobic conditions also induced the synthesis of the 106 000-, 90 000- and 70 000-molecular weight proteins (proteins 6, 2 and 3, respectively) (Fig 5.3)

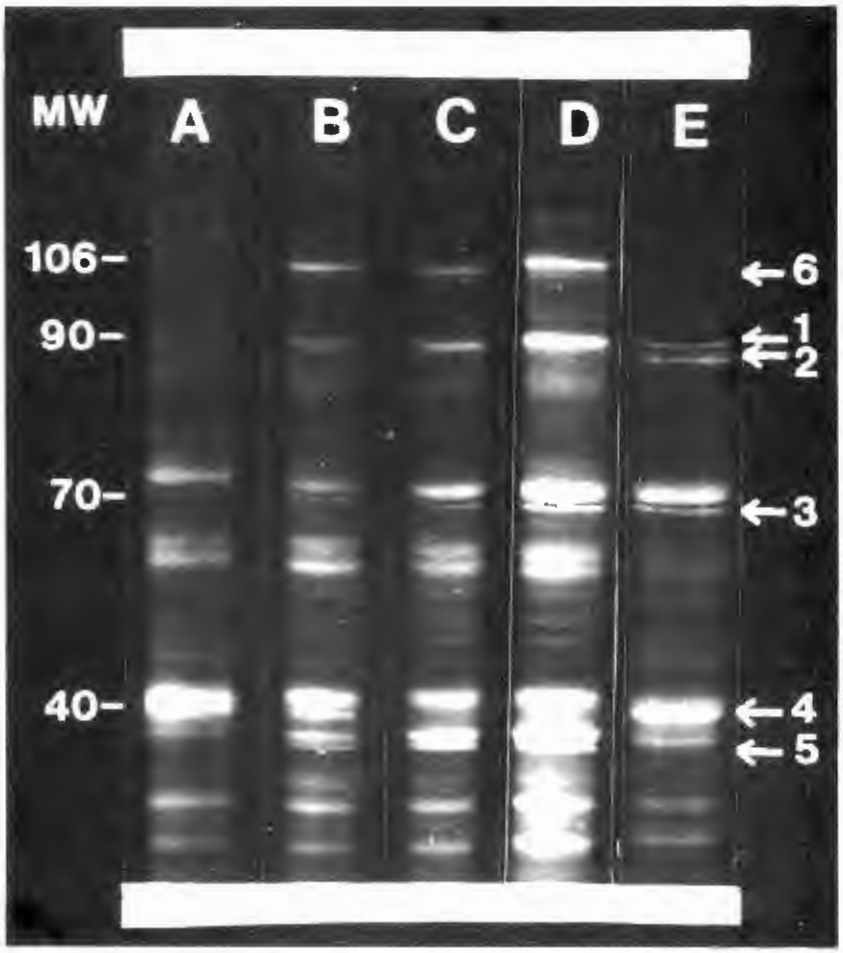

FIG 5.1 SDS-PAGE of extracts of *B.fragilis* cells exposed to oxygen. The lanes represent: anaerobic cells (A); cells exposed to oxygen for 10 min (B) and 25 min (C); aerobically irradiated cells exposed to oxygen for 30 min (D); and anaerobically irradiated cells labelled 15 min after irradiation (E). MW, Molecular weight (10^3). The arrows indicate the positions of the induced or inhibited proteins.

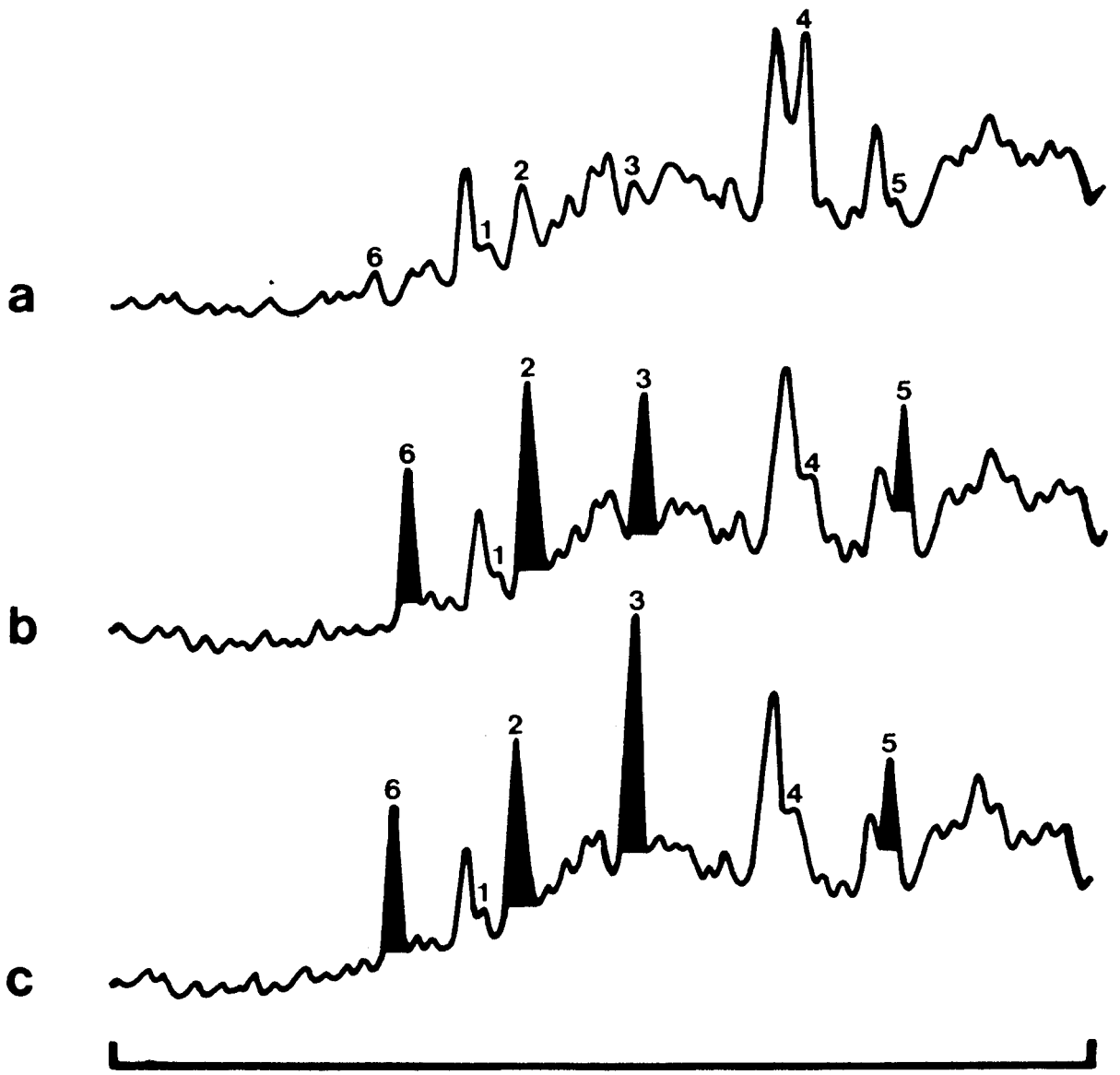

FIG 5.2 Densitometric comparison of [^{35}S] methionine-labelled protein bands after SDS-PAGE of extracts of anaerobic *B.fragilis* cells (a), cells exposed to oxygen for 25 min (b) and cells exposed to oxygen for 30 min after far-UV irradiation under aerobic conditions (c). Cells were irradiated to a survival level of 0,1% and cell samples were labelled for 10 min at 37°C. The induced proteins of molecular weights 106 000, 90 000, 70 000 and 37 000 are indicated by 6, 2, 3 and 5 respectively. The production of a protein with molecular weight of 40 000 (protein 4) is inhibited by oxygen.

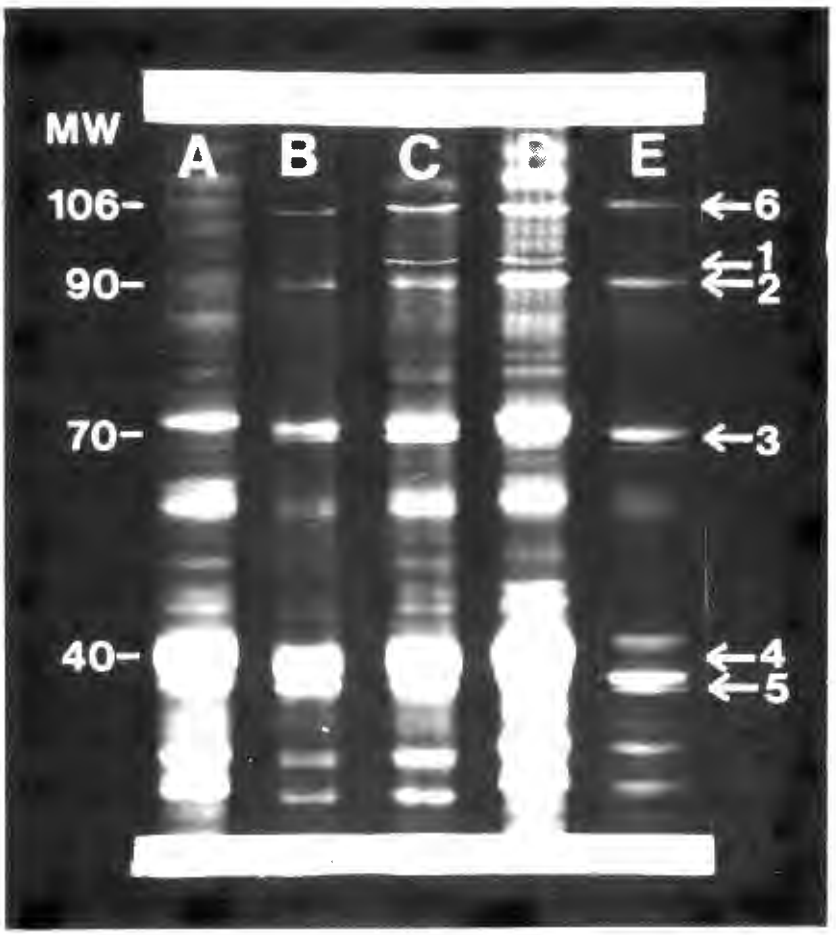

FIG 5.3 SDS-PAGE of extracts of *B.fragilis* cells treated with hydrogen peroxide. The lanes represent: anaerobic cells (A); anaerobic cells treated with hydrogen peroxide for 10 min (B) and 25 min (C); anaerobically irradiated cells treated with hydrogen peroxide for 25 min (D); cells exposed to oxygen and hydrogen peroxide for 25 min (E). MW, Molecular weight (10^3). The arrows indicate the positions of induced or inhibited proteins.

which were synthesized in small amounts in the absence of hydrogen peroxide. Hydrogen peroxide did not cause the induction of a 37 000-molecular weight protein (protein 5) and the level of the 40 000-molecular weight protein (protein 4) was not affected by hydrogen peroxide. However, hydrogen peroxide induced the synthesis of moderate levels of the 95 000-molecular weight protein (protein 1) which was also induced by UV irradiation under anaerobic conditions, but was absent in unirradiated anaerobic cells (Chapter 3; Schumann *et al*, 1982).

5.3.3 Oxygen and hydrogen peroxide treatment of cells

Simultaneous treatment of *B.fragilis* cells with oxygen and hydrogen peroxide resulted in the induced synthesis of the 106 000-, 90 000-, 70 000- and 37 000-molecular weight proteins (proteins 6, 2, 3 and 5, respectively), and the synthesis of the 40 000-molecular weight protein (protein 4) was inhibited (Fig 5.3). Although hydrogen peroxide induced a 95 000-molecular weight protein (protein 1) under anaerobic conditions, this protein (which was also induced by UV irradiation under anaerobic conditions) was not induced in cells exposed to oxygen and hydrogen peroxide.

5.3.4 Effect of oxygen and hydrogen peroxide on the induction of proteins by UV light

Irradiation of *B.fragilis* cells with far-UV light under aerobic conditions and reincubation of the irradiated cultures for 30 min in the presence of oxygen prior to pulse-labelling, resulted in the induction of the 90 000-molecular weight protein (protein 2) and the greatly enhanced synthesis of the 70 000-molecular weight protein (Figs 5.1 and 5.2). The 40 000-molecular weight protein (protein 4) was inhibited and the 37 000-molecular weight protein (protein 5) was only induced to low levels under these conditions (Fig 5.1).

Cells which were exposed to oxygen for 30 min and then returned to the anaerobic cabinet for 10 min before irradiation and incubation for a further 15 min before pulse-labelling, showed the induction of 95 000-molecular weight protein (protein 1) in addition to the 90 000- and 70 000-molecular weight proteins (proteins 2 and 3)(Fig 5.4). Although these cells had been exposed to oxygen prior to incubation and irradiation under anaerobic conditions, the synthesis of the 106 000- and 37 000-molecular weight proteins and the inhibition of the production of the 40 000-molecular weight protein was not observed.

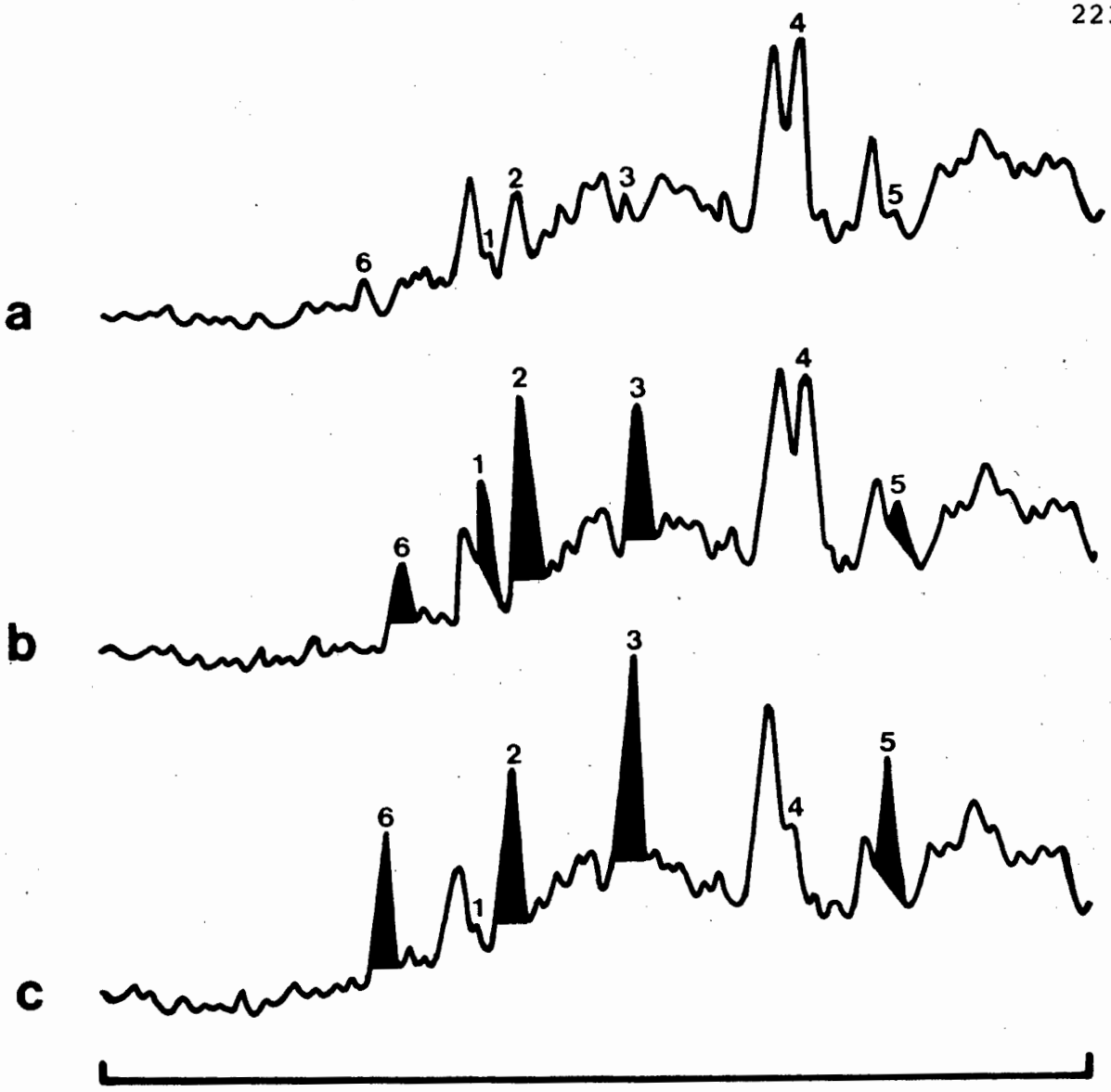

FIG 5.4 Densitometric comparison of [^{35}S] methionine-labelled protein bands after SDS-PAGE of extracts of anaerobic *B.fragilis* cells (a), cells irradiated under anaerobic conditions after exposure to oxygen for 30 min (b) and cells exposed to oxygen for 30 min after irradiation under aerobic conditions (c). Cells were irradiated to a survival level of 0,1% and cell samples were labelled for 10 min at 37°C. The induced proteins of molecular weights 106 000, 95 000, 90 000, 70 000 and 37 000 are indicated by 6, 1, 2, 3 and 5, respectively. The production of a protein with molecular weight of 40 000 (protein 4) is inhibited by oxygen.

The treatment of UV-irradiated cells with hydrogen peroxide under anaerobic conditions induced the synthesis of the 106 000-, 95 000-, 90 000- and 70 000-molecular weight proteins (proteins 6, 1, 2 and 3, respectively)(Fig 5.3). The levels of the 40 000- and 37 000-molecular weight proteins (proteins 4 and 5) were not affected by treatment of UV-irradiated cells with hydrogen peroxide under anaerobic conditions.

5.3.5 Effect of caffeine

The addition of sublethal concentrations of caffeine immediately after exposure of the cells to oxygen inhibited the induction of the 90 000- and 70 000-molecular weight proteins (proteins 2 and 3)(Fig 5.5). After treatment with caffeine the concentrations of the two proteins were similar to those in the unexposed cells. The addition of caffeine 25 min after exposure to oxygen, had no effect on the induction of the 90 000- and 70 000- molecular weight proteins (proteins 2 and 3). Caffeine did not prevent the induction by oxygen of the 106 000- and 37 000-molecular weight proteins (proteins 6 and 5), but there was a decrease in the concentration of the oxygen-induced 37 000-molecular weight protein after treatment with caffeine.

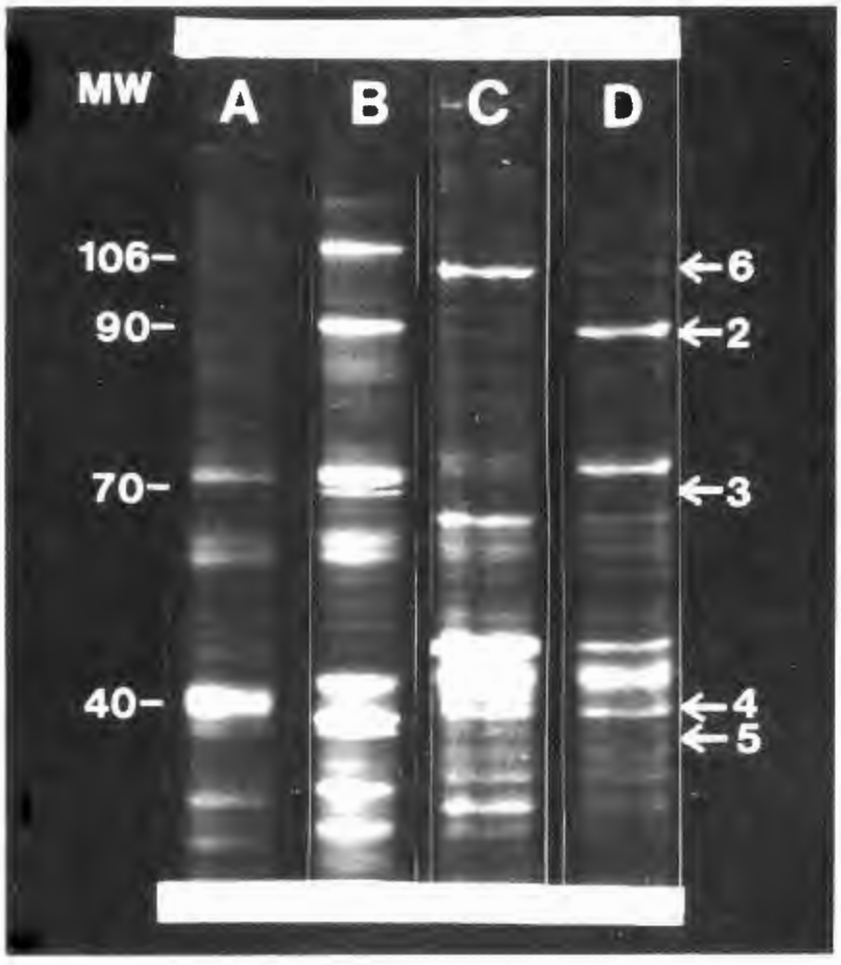

FIG 5.5 Effect of caffeine on the induction of proteins by oxygen in *B.fragilis*. The lanes represent SDS-PAGE extracts of: anaerobic cells (A); cells exposed to oxygen for 25 min (B); cells exposed to oxygen with caffeine (1 mg ml^{-1}) added immediately (C) and 25 min after exposure to oxygen (D). The cells in (C) were held for 25 min before labelling. MW, Molecular weight (10^3). The arrows indicate the positions of induced or inhibited proteins.

5.4 DISCUSSION

Exposure of exponential phase *B.fragilis* cells to oxygen induced two proteins (molecular weights of 90 000 and 70 000) which were also induced by treating the *B.fragilis* cells with hydrogen peroxide under anaerobic conditions. Previously we showed that far-UV irradiation of the cells resulted in the induction of the same 90 000- and 70 000-molecular weight proteins (Schumann *et al*, 1982). All three treatments are capable of damaging DNA (Beukers & Berends, 1960; Ananthaswamy & Eisenstark, 1977; Moody & Hassan, 1982) and all three have been shown to induce phage reactivation systems in *B.fragilis* (Slade *et al*, 1983a and 1983b) which differ in certain respects but may share common components. The addition of sublethal concentrations of caffeine to *B.fragilis* cells immediately after irradiation in the presence of oxygen prevented the induction of both the 90 000- and the 70 000-molecular weight proteins; caffeine also inhibited these two proteins after similar treatment under anaerobic conditions (Schumann *et al*, 1982). On a physiological response level caffeine reduces the survival of UV-irradiated *B.fragilis* cells (Jones *et al*, 1980) and hydrogen peroxide-induced phage reactivation (Slade *et al*, 1983b), and also completely inhibits oxygen-induced (Slade *et al*, 1983a) and UV-induced (J.R.Parker, personal communication) phage reactivation. These

results support the conclusion drawn in Chapter 3 that the 90 000- and 70 000-molecular weight proteins may be involved in excision repair processes in *B.fragilis*, and in addition suggest that these two proteins are closely associated with phage reactivation processes in this anaerobe.

It is interesting that although we reported the induction by oxygen of a host cell reactivation system in *B.fragilis* which enhanced the survival of irradiated phage (Slade *et al*, 1983a), this phage repair system is apparently unable to decrease the sensitivity of the cells to UV radiation under aerobic conditions. An analogous and seemingly contradictory situation has been shown to occur in *Haemophilus influenzae* (Notani & Setlow, 1980). A small dose of UV radiation followed by 30 min incubation produced maximum reactivation of UV irradiated phage, and pretreatment of cells with the low dose of UV radiation might be expected to make the cells less sensitive to subsequent UV doses. However, this did not occur and pretreatment with UV caused the *H.influenzae* cells to be more sensitive. Aeration of *B.fragilis* cells also leads to the cells being more sensitive to UV irradiation than they are under anaerobic conditions in the absence or presence of hydrogen peroxide (Jones *et al*, 1980; Slade *et al*, 1983b). Exposure of *B.fragilis* cells to oxygen inhibited the synthesis of the 95 000-molecular

weight protein which was induced by UV irradiation under anaerobic conditions (Schumann *et al*, 1982) and by the hydrogen peroxide treatment of *B.fragilis* cells under anaerobic conditions. Oxygen also inhibited the synthesis of a 40 000-molecular weight protein. This inhibition appears to be a specific effect of molecular oxygen, since the level of this enzyme was unaffected by hydrogen peroxide treatment and UV irradiation of the cells under anaerobic conditions. It is therefore tempting to speculate that the inhibition of synthesis of either one or both the 95 000- and the 40 000-molecular weight proteins by oxygen renders the cells more sensitive to UV light.

At this point nothing is known about the functions of these two gene products. Some observations may, however, indicate possible relationships. LHR in *B.fragilis* only occur in the presence of oxygen (Jones & Woods, 1981) i.e. in the absence of the 95 000- and 40 000-molecular weight proteins. In *E.coli* LHR is dependent on functional *uvr* and *pol* gene products (Ganesan & Smith, 1969; Tang & Patrick, 1977b) and is only observed in *recA* mutants Ganesan & Smith, 1968). The *E.coli recA*$^{+}$ protein has a molecular weight of *c* 40 000 (Gudas & Mount, 1977; McEntee & Epstein, 1977; Sancar *et al*, 1980). A protein with similar *recA*$^{+}$-type functions could, however, have a different molecular weight in *B.fragilis*. *RecA*$^{+}$

induction in *E.coli* is very much dependent on the generation of DNA degradation products (Gudas & Pardee, 1975; Oishi & Smith, 1978; Oishi *et al*, 1981). The DNA synthesis profile of *B.fragilis* cells exposed to oxygen or irradiation in the presence of oxygen, lacked the phase of extensive DNA degradation observed after UV irradiation under anaerobic conditions in the absence and presence of hydrogen peroxide (Chapter 4). The 95 000-molecular weight protein is not induced in *B.fragilis* cells in the presence of oxygen.

Although we did not assay for the production of specific radical scavenging enzymes, there is some doubt as to whether catalases were involved in our experimental system. Glucose has been shown to inhibit catalase production and induction in a number of *Bacteroides* species (Gregory *et al*, 1977b). In our experiments *B.fragilis* was grown and exposed to oxygen and hydrogen peroxide in a minimal medium containing glucose as sole carbon source. However, both oxygen and hydrogen peroxide (but not UV light) induced the 106 000-molecular weight protein and synthesis of this protein may be due to the oxygen radical, hydrogen peroxide. The catalase enzyme isolated from *B.distasonis* has a molecular weight of 250 000 (Gregory *et al*, 1977a). High levels of SOD are induced in various *Bacteroides* species by exposure to oxygen (Gregory *et al*, 1977a;

Tally *et al*, 1977; Gregory *et al*, 1978; Rolfe *et al*, 1978). The molecular weight of the inducible SOD in *B.distasonis* is 40 000 and in *E.coli* the inducible MnSOD consists of subunits with molecular weights of 40 000 (Gregory *et al*, 1977a; Fee, 1981). Oxygen specifically induced a 37 000-molecular weight protein in *B.fragilis* cells which was not induced by either hydrogen peroxide treatment or UV irradiation of the cells. It is therefore suggested that the 37 000-molecular weight protein in *B.fragilis* cells could be an inducible SOD protein.

These studies with the anaerobe *B.fragilis* showed the induction of specific proteins and the synthesis of many other proteins during pulse-labelling experiments in the presence of oxygen. It confirms our previous conclusion that *B.fragilis* Bf-2 differs from *B.thetaiotaomicron* in that protein synthesis is not immediately inhibited by oxygen. It is not clear as yet whether the ability of *B.fragilis* to continue synthesizing proteins in the presence of oxygen and to induce proteins involved in repair processes by exposure of the cells to oxygen, is linked to its greater oxygen tolerance in comparison with certain other strict anaerobes.

CHAPTER 6

SUMMARY AND CONCLUSION

It has become apparent in recent years that fundamental studies on the molecular genetics of anaerobes are important in furthering our understanding of the biochemistry, physiology and pathogenicity of anaerobes, as well as in realizing their potential in biotechnology.

Initial studies on the physiological response of the anaerobe *B.fragilis* to UV irradiation have shown that the recovery and repair processes occurring in *B.fragilis* cells after UV irradiation differ in a number of ways from those known to occur in wild type *E.coli* cells and other aerobic bacteria. These differences, and in particular the finding that *B.fragilis* differs from other bacteria in that far-UV light inactivation of the cells is dependent on the presence of oxygen, had triggered the research reported in this thesis. Previously it was shown that the decrease in survival of *B.fragilis* cells irradiated under aerobic conditions was not due to an increase in the amount of UV damage sustained by the DNA, but that the differences were rather due to a decreased repair potential of the cells under aerobic

conditions (Jones *et al*, 1980; Jones & Woods, 1981).

The demonstration of LHR and phage reactivation in *B. fragilis* after UV irradiation (Jones & Woods, 1981; Slade *et al*, 1983b) indicated that repair phenomena can be induced in this organism under anaerobic conditions. Exposure of *B.fragilis* cells to far-UV light under anaerobic conditions resulted in the induction of one novel protein (molecular weight 95 000) and the induced synthesis of two other proteins (molecular weights 90 000 and 70 000) which were synthesized in small amounts in unirradiated cells. UV survival and the induction of these three proteins was not affected by the addition of sodium arsenite to the cells immediately after irradiation. Sodium arsenite inhibits *recA*$^{+}$-dependent repair processes in irradiated *E.coli* cells (Rossman *et al*, 1975). However, the production of all three UV-inducible proteins was inhibited in the presence of sublethal concentrations of caffeine. Caffeine inhibits *uvr*$^{+}$-dependent excision repair processes in other organisms (Rupert & Harm, 1966; Fong & Bockrath, 1979; Rothman, 1980) and was previously also shown to inhibit several UV-induced repair phenomena in *B.fragilis* cells. It was thus suggested that the three inducible proteins may be involved in excision repair processes in *B.fragilis*.

DNA synthesis in prelabelled *B.fragilis* cells irradiated under anaerobic conditions was characterized by an extensive and rapid degradation of DNA immediately after irradiation, followed by net DNA synthesis. The extensive degradation masked the discovery, which was made with unlabelled cells, that DNA synthesis was decreased, but never completely inhibited by UV irradiation, and continued in a linear mode for a dose-dependent period before exponential synthesis resumed. In both the extensive degradation of UV-irradiated DNA and the continuation of DNA synthesis after irradiation, the *B.fragilis* DNA synthesis profile differed from that in wild type *E.coli* cells and was in fact similar to that in *E.coli recA* mutants (Clark, 1973; Trgovčević *et al*, 1980; Williams *et al*, 1981).

The degradation of UV-irradiated *B.fragilis* DNA was inhibited by chloramphenicol, suggesting that *de novo* protein synthesis is required for the observed degradation. The addition of caffeine to cells immediately after UV irradiation also inhibited the extensive DNA degradation in *B.fragilis*. In *E.coli* caffeine greatly reduces DNA degradation in *recA* mutants of this organism. The presence of chloramphenicol in cells immediately after UV irradiation inhibits the $recA^{+}$-dependent resumption of DNA synthesis in *E.coli* cells (Kogoma & Connaughton, 1978; Lark & Lark, 1978; Kogoma *et al*,

1979; Hall & Mount, 1981). In *B.fragilis* cells chloramphenicol reduced DNA synthesis but never completely inhibited it, and after a certain length of time exponential DNA synthesis resumed (at a rate similar to that in irradiated cells in the absence of the drug). The DNA synthesis in irradiated *B.fragilis* cells treated with chloramphenicol is analogous to abnormal stable UV-induced DNA replication in *E.coli* in that it could occur in the absence of protein synthesis (Kogoma & Lark, 1975; Lark & Lark, 1978) but *B.fragilis* differs from *E.coli* in that protein synthesis was not necessary immediately after irradiation for the initiation of stable DNA synthesis (Kogoma *et al*, 1979).

Excision repair processes can continue in the presence of chloramphenicol in irradiated *E.coli* cells (Swenson & Setlow, 1966). The addition of caffeine to UV-irradiated *B.fragilis* cells rapidly inhibited DNA synthesis and at the same time reduced the survival of irradiated *B.fragilis* cells. It is thus suggested that excision repair processes may play an important role in the repair of damaged DNA and consequent survival in UV-irradiated *B.fragilis* cells under anaerobic conditions. RNA and protein synthesis in *B.fragilis* and *E.coli* cells are less sensitive to UV than DNA synthesis.

Colony formation in *B.fragilis* cells was not immediately affected by UV irradiation. A transient increase in the number of colony-forming units was followed after a dose-dependent period of time by an inhibition of colony formation. Cell division in irradiated *B.fragilis* cultures did not resume during a further 2,5 h of anaerobic incubation, although DNA synthesis continued during this time.

B.fragilis is interesting in that although it is an obligate anaerobe it can be maintained in aerobic holding solutions for 1 to 6 hours without loss of viability (Jones & Woods, 1981). Exposure of unirradiated exponential *B.fragilis* cells to oxygen caused an inhibition of colony formation and a reduction in the rate of DNA synthesis, but DNA synthesis continued for a limited period of time before it was totally inhibited by oxygen. The rate and time of inhibition of DNA synthesis by oxygen was affected by the method of aeration. RNA and protein synthesis were less sensitive to oxygen than DNA synthesis.

Uptake experiments demonstrated that the plateau in DNA synthesis which occurred in aerated *B.fragilis* cells was not due to a shutoff of isotope transport and DNA turnover studies revealed that the plateau in fact represented an overall net inhibition of DNA synthesis.

Since the incorporation of isotopes into RNA and protein continued after DNA synthesis was inhibited, the inhibition of DNA synthesis was not due to the inhibition of RNA or protein synthesis by oxygen. Our results on the effect of air on macromolecular synthesis in *B.fragilis* differ from those reported by Glass *et al* (1979) with *B.thetaiotaomicron* and by Stevenson (1979) with *B.ruminicola*. In these anaerobes oxygen inhibits uptake of isotopes and macromolecular synthesis. No reason for the difference between the *B.fragilis* Bf-2 strain and the other two *Bacteroides* species is known, but it suggests that there are substantial differences between species of *Bacteroides* and their response to oxygen. The *B.fragilis* Bf-2 strain was first isolated in 1977 and has been regularly exposed to air during subculturing over the last six years which may account for its response to oxygen.

DNA synthesis resumed in an exponential fashion if *B. fragilis* cultures were restored to anaerobic conditions before the plateau in DNA synthesis was reached. Once DNA synthesis in aerated *B.fragilis* cells was inhibited, restoration of the cells to anaerobic conditions did not result in a resumption of DNA synthesis. There was an interesting correlation between inhibition of DNA synthesis and loss of viability in *B.fragilis* cells exposed to oxygen. However, cell death in this anaerobe

upon prolonged exposure to oxygen appears to be due to something more than just an inhibition of DNA synthesis. Treatment of *B.fragilis* cells with hydrogen peroxide under anaerobic conditions could likewise inhibit colony formation but loss of viability did not follow the observed hydrogen peroxide-induced inhibition of DNA synthesis.

Studies with the anaerobe *B.fragilis* showed the induction of certain proteins and the synthesis of many other proteins during pulse-labelling experiments in the presence of oxygen, which confirmed our previous conclusion that *B.fragilis* differs from *B.thetaiotaomicron* in that protein synthesis is not inhibited immediately by oxygen. Assays for oxygen radical scavenging enzymes were not undertaken, but a 106 000-molecular weight protein was induced by both oxygen and hydrogen peroxide, but not by UV radiation, which strongly suggested that it is a hydrogen peroxide-inducible protein. The hydrogen peroxide scavenging enzymes, catalase and peroxide, are inducible in *E.coli* K12 cells (Hassan & Fridovich, 1977a) and several *Bacteroides* species were previously shown to contain high levels of catalase. Another protein of molecular weight 37 000 was induced by oxygen, but not by hydrogen peroxide or UV radiation under anaerobic conditions, and it is suggested that this protein may be an

inducible SOD. The molecular weight of this *B.fragilis* protein is very similar to that of the inducible SOD in *B.distasonis* and *E.coli* (Gregory *et al*, 1977a; Fee, 1981).

Exposure of exponential phase *B.fragilis* cells to oxygen or to hydrogen peroxide treatment under anaerobic conditions, resulted in the induction of two proteins (90 000- and 70 000-molecular weight proteins) which were also induced by far-UV radiation under anaerobic conditions. All three agents have previously been shown to induce phage reactivation systems in *B.fragilis* (Slade *et al*, 1983a and 1983b). Caffeine inhibited the production of these two proteins under aerobic conditions (as well as under anaerobic conditions) and also inhibited or reduced oxygen- and hydrogen peroxide-induced phage reactivation. These findings, coupled with the fact that excision of pyrimidine dimers was shown to occur under both anaerobic and aerobic conditions (Jones & Woods, 1981), support the suggestion that the 90 000- and 70 000-molecular weight proteins are involved in excision repair and phage reactivation processes in *B.fragilis*. Two of the *E.coli uvr*$^{+}$ proteins were recently shown to be inducible by DNA damage (Fogliano & Schendel, 1981; Kenyon & Walker, 1981) and inducible excision repair is thought to be an important component of Weigle reactivation in *E.coli* cells (Cooper, 1981).

The induction of a phage reactivation system by oxygen does not protect *B.fragilis* cells against far-UV radiation and the presence of oxygen in fact sensitizes *B.fragilis* to far-UV radiation (Jones *et al*, 1980). This sensitization was found to be a specific effect of molecular oxygen since pretreatment of cells with hydrogen peroxide actually enhances the survival of the irradiated *B.fragilis* cells (Slade *et al*, 1983b). Exposure of *B.fragilis* cells to oxygen inhibited the induction of the 95 000-molecular weight protein which was induced by far-UV light and hydrogen peroxide treatment of the cells under anaerobic conditions. Oxygen also inhibited the synthesis of a 40 000-molecular weight protein which was unaffected by hydrogen peroxide treatment and UV radiation under anaerobic conditions. There is thus a possibility that the inhibition of synthesis of either one or both of the 95 000- and 40 000-molecular weight proteins by oxygen renders *B.fragilis* cells more sensitive to UV light.

DNA synthesis in *B.fragilis* was markedly affected by UV radiation and oxygen. Irradiation of *B.fragilis* in the presence of oxygen caused an immediate inhibition of DNA synthesis and the extensive degradation of DNA which occurred under anaerobic conditions immediately after

UV irradiation, was not observed in aerobically irradiated cells. RNA and protein synthesis were relatively unaffected under similar conditions and continued in the absence of net DNA synthesis. The pattern of DNA synthesis in cells treated with hydrogen peroxide after UV irradiation under anaerobic conditions, was similar to that in anaerobically irradiated cells in the absence of hydrogen peroxide. Since caffeine which has been shown to decrease the survival of irradiated *B.fragilis* cells, also inhibited DNA degradation and DNA synthesis in irradiated cells, it was suggested that the inhibition of DNA degradation and DNA synthesis by oxygen and UV radiation could be a reason for *B.fragilis* cells being more sensitive to UV radiation in the presence of oxygen.

Previous results on LHR in *B.fragilis* suggested that oxygen inhibits some aspects of "recombinational repair", resulting in sensitization to UV and the expression of LHR (Jones, 1979; Jones & Woods, 1981). The lack of success in obtaining chromosomal recombination in members of the *Bacteroidaceae*, along with the absence of lysogeny and the difficulties encountered in inducing mutants, also indicate some possible deficiency in the genetic recombination system in these organisms. Further studies on recombination processes in particular under the different conditions should thus be undertaken in

B.fragilis to supplement the basic studies on DNA repair reported in this thesis. Although it is not known whether *B.fragilis* can take up 5-bromouracil (BrdU), it would be worthwhile to try and pulse-label *B.fragilis* DNA in the presence of BrdU after UV irradiation under the different conditions, followed by isopycnic density distribution analysis of the denatured DNA. A density shift can be interpreted as evidence that extensive exchanges occur between parent DNA and the DNA made after irradiation. Preliminary experiments indicated that 50 and 100 $\mu g\ ml^{-1}$ of 5-BrdU had no effect on the growth of *B.fragilis* cells in minimal medium over 180 min (Slade, 1983) which suggested that 5-BrdU may not be taken up by *B.fragilis* cells.

The isolation of UV-sensitive and -resistant *B.fragilis* mutants, and in particular an excision repair-deficient mutant, is an essential prerequisite before any further characterization of post-replication repair is possible. A study of the rate of decrease of endonuclease-sensitive sites in such an excision repair mutant after irradiation under the different conditions, may help to establish whether the enhanced survival of cells irradiated under anaerobic conditions is due to a second mode or pathway of repair becoming operational under these conditions. In addition, repair-deficient mutants

would be extremely valuable in clarifying the functions of the different damage-inducible proteins in *B. fragilis* and explain their relationship with observed repair phenomena in this important anaerobe.

APPENDIX 1

MEDIA AND SOLUTIONS

1. Preparation of Anaerobic Media and Solutions

Prereduced and anaerobically sterilized (PRAS) media and solutions were prepared according to the methods of Moore (1966), Hungate (1969) and Holdeman and Moore (1972). Most of the oxygen in media was driven off by heating, and the media were further reduced by the addition of cysteine hydrochloride or sodium thioglycollate. They were then flushed with CO_2 and maintained in an anaerobic state in Astell roll tubes with tightly fitting rubber stoppers, Hungate tubes, or in anaerobic jars or boxes with silica gel in muslin bags and palladium-coated alumina pellets.

All sterilization was done by autoclaving at 121^{o}C for 15 min unless otherwise stated. Distilled deionized water was used in all media.

2. Anaerobic Media

2.1 Beef Liver Anaerobe Medium

Minced Beef Liver	500,0 g
Tap Water	1,0 ℓ

The beef liver was soaked overnight in the fridge and the fat skimmed off the top. The suspension was autoclaved for 10 min and filtered through cheese-cloth. The meat was saved.
To the liquid was added:

Peptone	10,0 g
K_2HPO_4	1,0 g

The pH was adjusted to 8,0 and the liquid was filtered again. The volume was made up to 1,0 litre with distilled water. A small amount of $CaCO_3$ was placed in each tube together with 1,5 cm of cooked meat. This mixture was then covered with broth to a total depth of 5 cm before the tubes with medium were autoclaved.

2.2 Brain Heart Infusion Broth

BHI	3,7 g
Yeast Extract	0,5 g
Sodium thioglycollate	0,11g
Na_2CO_3	0,4 g
Resazurin stock solution	1,0 mℓ
Distilled H_2O	100,0 mℓ

The medium was steamed for 30 min and 1,0 mℓ Haemin-Menadione stock solution was added. The broth was dispensed into Hungate tubes in 10 mℓ volumes, gassed immediately with CO_2 and autoclaved.

2.3 Brain Heart Infusion Agar

2.3.1 Plates

BHI	3,7 g
Yeast Extract	0,5 g
Agar (Difco)	1,5 g
Distilled H_2O	100,0 mℓ

The medium was autoclaved, cooled to 50°C and

Cysteine stock solution	1,0 mℓ
Na_2CO_3 stock solution	2,0 mℓ
Haemin-Menadione stock solution	1,0 mℓ

were added prior to pouring.

2.3.2 Slopes

BHI	3,7 g
Yeast Extract	0,5 g
Agar (Difco)	1,5 g
Distilled H_2O	100,0 mℓ

The medium was steamed for 30 min and

Cysteine stock solution	1,0 mℓ
Na_2CO_3 stock solution	2,0 mℓ
Haemin-Menadione stock solution	1,0 mℓ

were added. The medium was dispensed in 10 mℓ amounts in Astell roll tubes, gassed, clamped, autoclaved and sloped.

2.4 Cysteine Stock Solution

Cysteine hydrochloride	1,0 g
Distilled H_2O	20,0 mℓ

Autoclaved. 1 mℓ cysteine stock solution was added per 100 mℓ cooled medium prior to pouring to give a final concentration of 0,5 mg $m\ell^{-1}$

2.5 Sodium Carbonate Stock Solution

Na_2CO_3	20,0 g
Distilled H_2O	100,0 mℓ

Autoclaved. 2 mℓ of Na_2CO_3 stock was added per 100 mℓ medium to give a final concentration of 4 mg $m\ell^{-1}$.

2.6 Haemin-Menadione Stock Solution

2.6.1 Menadione Stock Solution

Menadione	100,0 mg
95% ethanol	20,0 mℓ

Filter-sterilized and stored at 4°C.

2.6.2 Haemin Stock Solution

Haemin	50,0 mg
1 N NaOH	1,0 mℓ
Distilled H_2O	100,0 mℓ

Autoclaved and stored at 4°C.

2.6.3 Haemin-Menadione Stock Solution

Menadione stock solution	1,0 mℓ
Haemin stock solution	100,0 mℓ

Stored at 4°C.

1 mℓ of Haemin-Menadione stock solution was added per 100 mℓ cooled medium to give a final haemin concentration of 5 μg $m\ell^{-1}$.

2.7 Resazurin Stock Solution

Resazurin	20,0 mg
Distilled H_2O	100,0 mℓ

1 mℓ of Resazurin stock solution was added per 100 mℓ to give a final concentration of 2 μg $mℓ^{-1}$

2.8 Minimal Medium Broth

2.8.1 Solutions for Minimal Medium Broth

2.8.1.1 Mineral Solution

KH_2PO_4	18,0 g
NaCl	18,0 g
$CaCl_2.2H_2O$	0,53g
$MgCl_2.6H_2O$	0,4 g
$MnCl_2.4H_2O$	0,2 g
$CoCl_2.6H_2O$	0,02g

Made up to 1000 mℓ with distilled H_2O. Stored over 10% chloroform at 4^oC.

2.8.1.2 $FeSO_4.7H_2O$ Stock Solution

$FeSO_4.7H_2O$	0,04g
Distilled H_2O	100,0 mℓ

Stored over 10% chloroform at 4^oC.

1 mℓ of the solution was added to 100 mℓ medium to give a final concentration of 4 μg $mℓ^{-1}$.

2.8.1.3 $(NH_4)_2SO_4$ Stock Solution

$(NH_4)_2SO_4$	0,159g
Distilled H_2O	200,0 mℓ

The solution was autoclaved and 5 mℓ was added per 100 mℓ medium.

2.8.1.4 Glucose Stock Solution

Glucose	10,0 g
Distilled H_2O	100,0 mℓ

The solution was autoclaved and 5 mℓ was added per 100 mℓ of medium to give a final concentration of 5 mg $m\ell^{-1}$.

2.8.1.5 Vitamin B12 Stock Solution

A stock solution of 1 µg $m\ell^{-1}$ was made up with sterile distilled water. Stored at 4°C. 0,5 mℓ was added per 100 mℓ of medium to give a final concentration of 0,005 µg $m\ell^{-1}$.

2.8.2 Minimal Medium Broth (Varel & Bryant, 1974)

Mineral solution	5,0 mℓ
$FeSO_4$ stock solution	1,0 mℓ
$(NH_4)_2SO_4$ stock solution	5,0 mℓ
Resazurin stock solution	1,0 mℓ
Sodium thioglycollate	0,11 g
Distilled H_2O	79,0 mℓ

The medium was autoclaved and

Glucose stock solution	5,0 mℓ
Vitamin B12 stock solution	0,5 mℓ
Haemin-Menadione stock solution	1,0 mℓ
Na_2CO_3 stock solution	2,0 mℓ

were added asceptically. The broth was dispensed into sterile Hungate tubes of 10 mℓ amounts and was gassed immediately using sterile needles.

2.9 Ringer Solution, one-quarter-strength

2.9.1 Anaerobic Ringer Solution

NaCl	2,25g
KCl	0,105g
$CaCl_2$	0,12g
$NaHCO_3$	0,05g
Distilled H_2O	1,0 ℓ

The solution was autoclaved, thoroughly perfused while still hot and allowed to stand in the anaerobic cabinet for at least 24 hours before it was used.

2.9.2 Prereduced Ringer Solution

NaCl	2,25g
KCl	0,105g
$CaCl_2$	0,12g
$NaHCO_3$	0,05g
Na_2CO_3	0,4 g
Sodium thioglycollate	0,11g
Resazurin stock solution	1,0 mℓ
Distilled H_2O	100,0 mℓ

The solution was steamed for 30 minutes, dispensed in 9 mℓ or 9,9 mℓ volumes, gassed and then autoclaved.

3. Solutions and buffers for SDS polyacrylamide gel electrophoresis

3.1 SDS Sample Buffer

Glycerol	10,0 g
β-2-Mercaptoethanol	5,0 mℓ
Sodium dodecyl sulphate (SDS)	2,3 g
Tris(hydroxymethyl)-aminomethane(Tris)	0,76g

Dissoled and made up to 100 mℓ with distilled water. The pH was adjusted to 6,8 with concentrated HCl.

3.2 Acrylamide Stock Solution

Acrylamide (Merck)	29,2 g
N, N'-methylene-bisacrylamide	0,8 g
Distilled H_2O	100,0 mℓ

Dissolved and stored at $4^{o}C$ in the dark. The stock solution was discarded if not used within 3 days. Before the acrylamide stock solution was used, 2 to 3 spatulas full of Amberlite Resin CG-50, type II, 200 mesh (chromatographic grade) was added to it. This suspension was allowed to adsorb for at least 6 h before the resin was filtered off and the stock solution used in making up polyacrylamide gels.

3.3 Upper Gel Buffer

Tris (0,5M)	6,06g
SDS	0,4 g

Made up to 100 mℓ with distilled water. The pH was adjusted to 6,8 with conc. HCl

3.4 Lower Gel Buffer

Tris (1,5M)	18,18g
SDS	0,4 g

Made up to 100 mℓ with distilled water. The pH was adjusted to 8,8 with conc. HCl

3.5 Ammonium Persulphate Stock Solution

$(NH_4)_2S_2O_8$	1,0 g
Distilled H_2O	10,0 mℓ

This solution was freshly prepared for each set of polyacrylamide gels.

3.6 Polyacrylamide Gel Mixtures Quantity for 2 gels of 8,4%

3.6.1 Stacking Gel Mix

Acrylamide stock solution	2,0 mℓ
Upper gel buffer	3,0 mℓ
Distilled H_2O	7,0 mℓ

This was briefly degassed and

Ammonium persulphate stock	64 µℓ
TEMED	13 µℓ

was then added.

3.6.2 Resolving Gel Mix

Acrylamide stock solution	12,0 mℓ
Lower gel buffer	8,2 mℓ
Distilled H_2O	13,65 mℓ

The mixture was briefly degassed and

Ammonium persulphate stock	160 µℓ
TEMED	18 µℓ

was then added.

If the gels were not poured immediately, the gel mixtures were kept on ice until required. Polymerization commonly took 3 h to complete, but the gels were usually left overnight covered with plastic to prevent dehydration. Before the stacking gel was cast, the upper gel spacer was removed and any unpolymerized acrylamide washed away with lower gel buffer.

3.7 Running Buffer

Tris	16,25g
Glycine	71,5 g
SDS	5,0 g

Dissolved and made up to 5 litres with distilled water.

Stored at room temperature in the dark.

3.8 Destaining Solution

Glacial acetic acid	90,0 mℓ
Propan-2-ol	300,0 mℓ
Distilled H_2O	810,0 mℓ

Stored at room temperature.

3.9 Coomassie Blue Staining Solution 0,05%

Coomassie brilliant blue R250 0,25g

Dissolved in 10 mℓ absolute alchohol and filtered into 500 mℓ destaining solution. Stored at room temperature in the dark.

REFERENCES

ACHEY, P.M. and V.G. WHITFIELD. 1968. Influence of anoxia on radiation-induced breaks in the *Escherichia coli* chromosome. J. Bacteriol. 95: 1180-1181.

ACHEY, P. and H. DURYEA. 1974. Production of DNA strand breaks by the hydroxyl radical. Int. J. Radiat. Biol. 25: 595-601.

ADAMS, G.E., M.S. COOKE and B.D. MICHAEL. 1968. Rapid mixing in radiobiology. Nature 219: 1368-1369.

ADAMS, G.E. and M.S. COOKE. 1969. Electron-affinic sensitization.I. A structural basis for chemical radiosensitizers in bacteria. Int. J. Radiat. Biol. 15: 457-471.

ADAMS, G.E., R.H. BISBY, R.B. CUNDALL and R.L. WILLSON. 1971. On the mechanism of the radiation-induced inactivation of lysozyme in dilute aqueous solution. Int. J. Radiat. Biol. 20: 405-415.

ADAMS, G.E. and J.L. REDPATH. 1974. Selective free-radical reactions with proteins and enzymes: Pulse radiolysis and inactivation studies on papain. Int. J. Radiat. Biol. 25: 129-138.

ADLER, H.I. 1963. Catalase, hydrogen peroxide, and ionizing radiation. Radiat. Res. Suppl. 3: 110-129.

AHNSTRÖM, G. 1974. Repair processes in germinating seeds: caffeine enhancement of damage induced by gamma-radiation and alkylating chemicals. Mutat. Res. 26: 99-103.

ALEXANDER, R. 1957. Effect of oxygen on inactivation of trypsin by the direct action of electrons and alpha particles. Radiat. Res. 6: 653-660.

ALPER, T. 1954. The inactivation of free bacteriophage by irradiation and by chemical agents. J. Gen. Microbiol. 11: 313-324.

ALPER, T. 1956. The modification of damage caused by primary ionization of biological targets. Radiat. Res. 5: 573-586.

ALPER, T. 1968. Low oxygen enhancement ratios for radiosensitive bacterial strains, and the probable interaction of two types of primary lesions. Nature 217: 862-863.

ALPER, T. and N.E. GILLIES. 1960. The relationship between growth and survival after irradiation of *Escherichia coli* strain B and two resistant mutants. J. Gen. Microbiol. 22: 113-128.

ANANTHASWAMY, H.N. and A. EISENSTARK. 1976. Near-UV-induced breaks in phage DNA: sensitization by hydrogen peroxide (a tryptophan photoproduct). Photochem. Photobiol. 24: 439-442.

ANANTHASWAMY, H.N. and A. EISENSTARK. 1977. Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. J. Bacteriol. 130: 187-191.

ANDERS, R.F., D.M. HOGG and G.R. JAGO. 1970. Formation of hydrogen peroxide by group N streptococci and its effect on their growth and metabolism. Appl. Microbiol. 19: 608-612.

ANDERSON, J.D. and R.B. SYKES. 1973. Characterization of a β-lactamase obtained from a strain of *Bacteroides fragilis* resistant to β-lactam antibiotics. J. Med. Microbiol. 6: 201-206.

ARAGÃO, B.R.V., A.C. ARAÚJO and R.A. GOMES. 1980. Simplified fluctuation test to distinguish liquid holding recovery from cell multiplication in ultraviolet-irradiated *Escherichia coli*. J. Bacteriol. 144: 1176-1178.

ARBIGE, M. and W. CHESBRO. 1982. *relA* and related loci are growth rate determinants for *Escherichia coli* in a recycling fermenter. J. Gen. Microbiol. 128: 693-703.

ARCHIBALD, F.S. and I. FRIDOVICH. 1981. Manganese, superoxide dismutase and oxygen tolerance in some lactic acid bacteria. J. Bacteriol. 146: 928-936.

ASADA, K., K. YOSHIKAWA, M. TAKAHASHI, Y. MAEDA and K. ENMANJI. 1975. Superoxide dismutases from a blue-green alga *Plectonema boryanum*. J. Biol. Chem. 250: 2801-2807.

ASHLEY, N.V. and J.G. SHOESMITH. 1977. Continuous culture of *Clostridium sporogenes* and *Clostridium bifermentans* in the presence of oxygen. Proc. Soc. Gen. Microbiol. 4: 144.

BADWEY, J.A. and M.L. KARNOVSKY. 1980. Active oxygen species and the functions of phagocytic leukocytes. Annu. Rev. Biochem. 49: 695-726.

BAGG, A., C.J. KENYON and G.C. WALKER. 1981. Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 78: 5749-5753.

BAILONE, A., A. LEVINE and R. DEVORET. 1979. Inactivation of prophage λ repressor in vivo. J. Mol. Biol. 131: 553-572.

BALUCH, J., J.W. CHASE and R. SUSSMAN. 1980. Synthesis of *recA* protein and induction of bacteriophage lambda in single-strand deoxyribonucleic acid-binding protein mutants of *Escherichia coli*. J. Bacteriol. 144: 489-498.

BANNISTER, J.V. and W.H. BANNISTER. 1981. What is superoxide dismutase? Biochemical Education 9: 42-45.

BARNER, H.D. and S.S. COHEN. 1956. The relation of growth to the lethal damage induced by ultraviolet irradiation in *Escherichia coli*. J. Bacteriol. 71: 149-158.

BAYLISS, C.E. and W.M. WAITES. 1979. The synergistic killing of spores of *Bacillus subtilis* by hydrogen peroxide and ultraviolet light irradiation. FEMS Microbiol. Lett. 5: 331-333.

BEAUCHAMP, C. and I. FRIDOVICH. 1970. A mechanism for the production of ethylene from methional. J. Biol. Chem. 245: 4641-4646.

BEAUCHAMP, C. and I. FRIDOVICH. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

BEAUCHAMP, C. and I. FRIDOVICH. 1973. Isozymes of superoxide dismutase from wheat germ. Biochim. Biophys. Acta 317: 50-64.

BENBOUGH, J.E. 1969. Factors affecting the toxicity of oxygen towards airborne coliform bacteria. J. Gen. Microbiol. 56: 241-250.

BEN-HUR, E. and R. BEN-ISHAI. 1968. Trans-syn thymine dimers in ultraviolet-irradiated denatured DNA: identification and photoreactivability. Biochim. Biophys. Acta 166: 9-15.

BERG, J-O., L. LINDQVIST and C.E. NORD. 1980. Purification of glycoside hydrolases from *Bacteroides fragilis*. Appl. Environ. Microbiol. 40: 40-47

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY. 1974. 8th edn., edited by R.E. Buchanan and N.E. Gibbons. The Williams and Wilkins Co., Baltimore.

BERNSTEIN, C. 1981. Deoxyribonucleic acid repair in bacteriophage. Microbiol. Rev. 45: 72-98.

BEUKERS, R. and W. BERENDS. 1960. Isolation and identification of the irradiation product of thymine. Biochim. Biophys. Acta 41: 550-551.

BIELSKI, B.H.J. and P.C. CHAN. 1976. Re-evaluation of the kinetics of lactate dehydrogenase-catalyzed chain oxidation of nicotinamide adenine dinucleotide by superoxide radicals in the presence of ethylenediaminetetraacetate. J. Biol. Chem. 251: 3841-3844.

BILLEN, D. 1967. Modification of the release of cellular constituents by irradiated *E.coli*. Arch. Biochem. Biophys. 67: 333-340.

BILLEN, D. 1969. Replication of the bacterial chromosome: location of new initiation sites after irradiation. J. Bacteriol. 97: 1169-1175.

BILLEN, D. and L.B. CARREIRA. 1971. Characterization of DNA synthesis in an *Hcr* mutant of *Escherichia coli* exposed to ultraviolet light. Microbios 3: 153-163.

BLOK, J. and W.S.D. VERHEY. 1968. The attack of free radicals on biologically active DNA in irradiated aqueous solution. Radiat. Res. 34: 689-703.

BOCKRATH, R.C. and P.C. HANAWALT. 1980. Ultraviolet light induction of *recA* protein in a *recB uvrB* mutant of *Escherichia coli*. J. Bacteriol. 143: 1025-1028.

BOERI, E. and R.K. BONNICHSEN. 1952. Oxidation of thiol groups by catalase. Acta Chem. Scand. 6: 968-970.

BÖHME, H. 1963. Mutagen stability of *Proteus mirabilis* against UV- and X-irradiation. Microbial Genetics Bulletin 19: 5.

BOMBERG, H.J. and K.A. LUSE. 1963. Resonance radiation effects of low energy monochromatic X-rays on the metalloenzyme catalase. (Abstract). Radiat. Res. 19: 212.

BONURA, T. and K.C. SMITH. 1975. Enzymatic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K-12. J. Bacteriol. 121: 511-517.

BOOTH, S.J., R.L. VAN TASSEL, J.L. JOHNSON and T.D. WILKINS. 1979. Bacteriophages of *Bacteroides*. Rev. Infect. Dis. 1: 325-336.

BOYCE, R.P. and P. HOWARD-FLANDERS. 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E.coli* K-12. Proc. Natl. Acad. Sci. USA 51: 293-300.

BOYCE, R.P. and M. TEPPER. 1968. X-ray induced single-strand breaks and joining of broken strands in superinfecting λ DNA in *Escherichia coli* lysogenic for λ. Virology 34: 344-351.

BOYE , E., I. JOHANSEN and T. BRUSTAD. 1974. Time scale for rejoining of bacteriophage λ deoxyribonucleic acid molecules in superinfected *pol*$^+$ and *polA1* strains of *Escherichia coli* after exposure to 4 MeV electrons. J. Bacteriol. 119: 522-533.

BOYLE, J.R. and R.B. SETLOW. 1970. Correlations between host-cell reactivation, ultraviolet reactivation and pyrimidine dimer excision in the DNA of bacteriophage λ. J. Mol. Biol. 51: 131-144.

BOYLE, J.M. and P.A. SWENSON. 1971. Loss of the capacity of UV-irradiated *Escherichia coli* B/r to grow T4D. Virology 44: 37-45.

BRAUN, A. and L.GROSSMAN. 1974. An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from the *uvrA* and *uvrB* mutants. Proc. Natl. Acad. Sci. USA 71: 1838-1842.

BRAUN, A.G., M. RADMAN and L. GROSSMAN. 1976. Enzymatic repair of DNA : sites of hydrolysis by the *Escherichia coli* endonuclease specific for pyrimidine dimers (correndonuclease II). Biochemistry 15: 4116-4120.

BRENT, R. and M. PTASHNE. 1980. The *lexA* gene product represses its own promoter. Proc. Natl. Acad. Sci. USA 77: 1932-1936.

BRENT, R. and M. PTASHNE. 1981. Mechanism of action of the *lexA* gene product. Proc. Natl. Acad. Sci. USA 78: 4204-4208.

BRESLER, S.E., I. YU GORYSHIN and V.A. LANZOV. 1981. The process of general recombination in *Escherichia coli* K12. Structure of intermediate products. Mol. Gen. Genet. 183: 139-143.

BREWER, D.G., S.E. MARTIN and Z.J. ORDAL. 1977. Beneficial effects of catalase or pyruvate in a most-probable-number technique for the detection of *Staphylococcus aureus*. Appl. Environ. Microbiol. 34: 797-800.

BRIDGES, B.A. 1972. Evidence for a further dark repair process in bacteria. Nat. New. Biol. 240: 52-53.

BRIDGES, B.A. 1978. The involvement of *E.coli* DNA polymerase III in constitutive and inducible mutagenic repair, p.345-348. *In* P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), *DNA repair mechanisms*. Academic Press, New York.

BRIDGES, B.A. and R.P. MOTTERSHEAD. 1978. Mutagenic DNA repair in *Escherichia coli*. VII. Constitutive and inducible manifestations. Mutat. Res. 52: 151-159.

BROWN, D.E. 1970. Aeration in the submerged culture of micro-organisms, p.125-174. *In* J.R. Norris and D.W. Ribbons (eds.), *Methods in Microbiology*, Vol.2. Academic Press, London.

BROWN, O.R. 1975. Failure of lipoic acid to protect against acute cellular oxygen toxicity in *Escherichia coli*. Microbios 14: 205-217.

BROZMANOVÁ, J. and M. SEDLIAKOVÁ. 1980. Effect of thymine deprivation on the restoration of DNA synthesis in ultraviolet-irradiated *Escherichia coli* B/r *Hcr*$^{+}$. J. Gen. Microbiol. 119: 527-533.

BRUCE, A.K. 1958. Response of potassium retentivity and survival of yeast to far-ultraviolet, near-ultraviolet and visible, and X-radiation. J. Gen. Physiol. 41: 693-701.

BRUNSCHEDE, H. and H. BREMER. 1969. Protein synthesis in *Escherichia coli* after irradiation with ultraviolet light. J. Mol. Biol. 41: 25-38.

BRUSTAD, T. 1966. The effects of radical scavengers on the radiosensitivity of lysozyme in dilute aqueous solutions of varying pH. Radiat. Res. 27: 456-473.

BRUSTAD, T. 1968. Rapid-mixing and pulse radiolysis equipment as tools in radiobiological research. Scand. J. Clin. Lab. Invest. 22: 31-40.

BRUYNINCKX, W.J., H.S. MASON and S.A. MORSE. 1978. Are physiological oxygen concentrations mutagenic? Nature 274: 606-607.

BRYANT, P.E. 1976. Absence of oxygen effect for induction of resistance to ionising radiation. Nature 261: 588-590.

BUKHARI, A.I. and D. ZIPSER. 1973. Mutants of *Escherichia coli* with a defect in the degradation of nonsense fragments. Nature (London) New Biol. 243: 238-241.

BURT, S.J. and D.R. WOODS. 1976. R factor transfer to obligate anaerobes from *Escherichia coli*. J. Gen. Microbiol. 93: 405-409.

CADET, J. and R. TEOULE. 1978. Comparative study of oxidation of nucleic acid components by hydroxyl radicals, singlet oxygen and superoxide anion radicals. Photochem. Photobiol. 28: 661-667.

CAMPBELL, J.E. and R.L. DIMMICK. 1966. Effect of 3% H_2O_2 on the viability of *Serratia marcescens*. J. Bacteriol. 91: 925-929.

CANCELLIERE, G., P. GIACCHI, P. MISITI-DORELLO and M. QUINTILIANI. 1975. The influence of agents that enhance lethal effects of radiation on the damage to bacterial membranes by X rays and ultraviolet light. Radiat. Res. 64: 593-601.

CARLSSON, J., J. WRETHEN and G. BECKMAN. 1977. Superoxide dismutase in *Bacteroides fragilis* and related *Bacteroides* species. J. Clin. Microbiol. 6: 280-284.

CARLSSON, J., G. NYBERG and J. WRETHEN. 1978. Hydrogen peroxide and superoxide radical formation in anaerobic broth media exposed to atmospheric oxygen. Appl. Environ. Microbiol. 36: 223-229.

CARLSSON, J., G.P.D. GRANBERG, G.K. NYBERG and M.K. EDLUND. 1969. Bactericidal effect of cysteine exposed to atmospheric oxygen. Appl. Environ. Microbiol. 37: 383-390.

CARLSSON, J. and V.S. CARPENTER. 1980. The *recA*$^+$ gene product is more important than catalase and superoxide dismutase in protecting *Escherichia coli* against hydrogen peroxide toxicity. J. Bacteriol. 142: 319-321.

CASAREGOLA, S., R.D'ARI and O. HUISMAN. 1982a. Role of DNA replication in the induction and turn-off of the SOS response in *Escherichia coli*. Mol. Gen. Genet. 185: 440-444.

CASAREGOLA, S., R. D'ARI and O. HUISMAN. 1982b. Quantitative evaluation of *recA* gene expression in *Escherichia coli*. Mol. Gen. Genet. 185: 430-439.

CASHEL, M. 1975. Regulation of bacterial ppGpp and pppGpp. Annu. Rev. Microbiol. 29: 301-318.

CASHEL, M. and J. GALLANT. 1974. Cellular regulation of guanosine tetraphosphate and guanosine pentaphosphate, p.733-745. *In* M. Nomura, A. Tissures and P. Lengyel (eds.), *Ribosomes*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

CASTELLAZZI, M., M. JACQUES and J. GEORGE. 1980. *tif*-stimulated deoxyribonucleic acid repair in *Escherichia coli* K-12. J. Bacteriol. 143: 703-709.

CHASE, J.W. and C.C. RICHARDSON. 1974. Exonuclease VII of *Escherichia coli* : mechanism of action. J. Mol. Biol. 249: 4553-4561.

CHUNG, C.H. and A.L. GOLDBERG. 1981. The product of the *lon (capR)* gene in *Escherichia coli* is the ATP-dependent protease, protease La. Proc. Natl. Acad. Sci. USA 78: 4931-4935.

CLARK, A.J. 1973. Recombination deficient mutants of *E.coli* and other bacteria. Annu. Rev. Genet. 7: 67-86.

CLARK, A.J. 1980. A view of the *recBC* and *recF* pathways of *E.coli* recombination, p.891-899. *In* B. Alberts (ed.), *Mechanistic studies of DNA replication and genetic recombination*. ICN-UCLA Symp. XIX.

CLARK, A.J., M. CHAMBERLIN, R.P. BOYCE and P. HOWARD-FLANDERS. 1966. Abnormal metabolic response to ultra-violet light of a recombination-deficient mutant of *Escherichia coli* K-12. J. Mol. Biol. 19: 442-454.

CLARK, A.J. and M.R. VOLKERT. 1978. A new classification of pathways repairing pyrimidine dimer damage in DNA, p.57-72. *In* R.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), *DNA repair mechanisms*. Academic Press, New York.

CLARKE, L. and J. CARBON. 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *Escherichia coli* genome. Cell 9: 91-99.

COLLINSON, E., F.S. DAINTON and B. HOLMES. 1950. Inactivation of ribonuclease in dilute aqueous solutions. Inactivation by hydroxyl radicals. Nature 165: 267-269.

COOPER, P.K. 1981. Inducible excision repair in *Escherichia coli*, p.139-146. *In* E. Seeberg and K. Kleppe (eds.), *Chromosome damage and Repair*. Plenum Publishing Corp., New York.

COOPER, P.K. and P.C. Hanawalt. 1972a. Heterogeneity of patch size in repair replicated DNA in *Escherichia coli*. J. Mol. Biol. 67: 1-10.

COOPER. P.K. and P.C. HANAWALT. 1972b. Role of DNA polymerase I and the *rec* system in excision repair in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 69: 1156-1160.

COOPER, P.K. and J.G. HUNT. 1978. Alternative pathways for excision and resynthesis in *Escherichia coli*: DNA polymerase III role?, p.255-260. *In* P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), *DNA repair mechanisms*. Academic Press, New York.

COX, C.S., M.C. BONDURANT and M.T. HATCH. 1971. Effects of oxygen on aerosol survival of radiation sensitive and resistant strains of *Escherichia coli*. B.J.Hyg. 69: 661-672.

COX, M., K. McENTEE and R. LEHMAN. 1981. A simple and rapid procedure for the large-scale purification of the *recA* protein from *Escherichia coli*. J. Biol. Chem. 256: 4676-4678.

CRAIG. N.L. and J.W. ROBERTS. 1980. *E.coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. Nature 283: 26-30.

CRAIG, N.L. and J.W. ROBERTS. 1981. Function of nucleoside triphosphate and polynucleotide in *Escherichia coli recA* protein-directed cleavage of phage λ repressor. J. Biol. Chem. 256: 8039-8044.

D'ARI, R., J. GEORGE and O. HUISMAN. 1979. Suppression of *tif*-mediated induction of SOS functions in *Escherichia coli* by an altered *dnaB* protein. J. Bacteriol. 140: 381-387.

D'ARI, R. and O. HUISMAN. 1982. DNA replication and indirect induction of the SOS response in *Escherichia coli*. Biochimie 64: 623-627.

DAVIES, D.J.G. 1968. Effect of water on the kinetics of the postirradiation oxygen effect, p.235-238. Annual report, Division of Biological and Medical Research. Argonne National Laboratory, Argonne, Illinois.

DeBOER, H., A. BAKKER, W. WEYER and M. GRUBER. 1975. The role of energy generating processes in the degradation of guanosine tetraphosphate, ppGpp, in *Escherichia coli*. Biochim Biophys. Acta 432: 361-368.

DEFAIS, M., P. CAILLET-FAUQUET, M.S. FOX and M. RADMAN. 1976. Induction kinetics of mutagenic DNA repair activity in *E.coli* following ultraviolet irradiation. Mol. Gen. Genet. 148: 125-130.

DELIHAS, N. 1961. The ability of irradiated bacteriophage T2 to initiate the synthesis of deoxycytidylate hydroxymethylase in *Escherichia coli*. Virology 13: 242-248.

DEVORET, R. 1981. Inducible error-prone repair and induction of prophage lambda in *Escherichia coli*. Prog. Nucl. Acid Res. Mol. Biol. 26: 251-263.

DIRKSEN, M., J.S. WIBERG, J.F. KOERNER and J.M. BUCHANAN. 1960. Effect of ultraviolet irradiation of bacteriophage T2 on enzyme synthesis in host cells. Proc. Natl. Acad. Sci. USA 46: 1425-1430.

DOLIN, M.I. 1959. Oxidation of reduced diphosphopyridine nucleotide by *Clostridium perfringens*. 1. Relation of peroxide to the overall reaction. J. Bacteriol. 77: 383-392.

DOLIN, M.I. 1977. DPNH peroxidase: effector activities of DPN^+. Biochem. Biophys. Res. Commun. 78: 393-400.

DOMON, M., B. BURTON, A. PORTE and A.M. RAUTH. 1970. The interaction of caffeine with ultra-violet-light-irradiated DNA. Int. J. Radiat. Biol. 17: 395-399.

DOUDNEY, C.O. 1959. Macromolecular synthesis in bacterial recovery from ultraviolet light. Nature 184: 189-190.

DOUDNEY, C.O. 1960. Inhibition of nucleic acid synthesis by chloramphenicol in synchronized cultures of *Escherichia coli*. J. Bacteriol. 79: 122-124.

DOUDNEY, C.O. 1965. Ultraviolet light effects on deoxyribonucleic acid replication, p.120-141. In *Cellular radiation biology*, 18th Symposium on Fundamental Cancer Research. The Williams & Wilkins Co., Baltimore.

DOUDNEY, C.O. 1966. Requirement for ribonucleic acid synthesis for deoxyribonucleic acid replication in bacteria. Nature 211: 39-41.

DOUDNEY, C.O. 1968. Ultraviolet light effects on the bacterial cell. Curr. Top. Microbiol. Immunol. 46: 116-175.

DOUDNEY, C.O. 1971. Increased resistance of DNA replication to ultraviolet light damage in amino acid-starved bacteria. Biochim. Biophys. Acta 228: 57-66.

DOUDNEY, C.O. 1973. Rifampicin limitation of DNA synthesis in ultraviolet damaged bacteria: evidence for postirradiation replication synchrony. Biochim. Biophys. Acta 312: 243-247.

DOUDNEY, C.O., B.F. WHITE and B.J. BRUCE. 1964. Acriflavin modification of nucleic acid formation, mutation induction and survival in ultraviolet light exposed bacteria. Biochem. Biophys. Res. Commun. 15: 70-75.

DRAKULIĆ, M. and M. ERRERA. 1959. Chloramphenicol-sensitive DNA synthesis in normal and irradiated bacteria. Biochim. Biophys. Acta 31: 459-463.

DRAKULIĆ, M., E. KOS and B. BRDAR. 1966. Comparison of survival of UV-irradiated uracil-requiring strain of *Escherichia coli* B in the presence of chloramphenicol and in the absence of the essential metabolite. Photochem. Photobiol. 5: 265-267.

DROZD, J. and J.R. POSTGATE. 1970. Effects of oxygen on acetylene reduction, cytochrome content and respiratory activity of *Azotobacter chroococcum*. J. Gen. Microbiol. 63: 63-73.

EBINA, Y., F. KISHI, T. MIKI, H. KAGAMIYAMA, T. NAKAZAWA and A. NAKAZAWA. 1981. The nucleotide sequence surrounding the promoter region of colicin E1 gene. Gene 15: 119-126.

EHRET, C.F., B. SMALLER, E.L. POWERS and R.B. WEBB. 1960. Thermal annealment and nitric oxide effects on free radicals in X-irradiated cells. Science 132: 1768-1769.

EISENSTARK, A. 1977. Genetic recombination in bacteria. Annu. Rev. Genet. 11: 369-396.

EMMERSON, P.T. 1968. Recombination-deficient mutants of *Escherichia coli* that map between *thyA* and *argA* Genetics 60: 19-30.

EMMERSON, P.T. and S.C. WEST. 1977. Identification of protein X of *Escherichia coli* as the $recA^{+}/tif^{+}$ gene product. Mol. Gen. Genet. 155: 77-85.

EPP, E.R., H. WEISS, N.D. KESSARIS, A. SANTOMASSO, J. HESLIN and C.C. LING. 1973. Oxygen diffusion times in bacterial cells irradiated with high-intensity pulsed electrons: new upper limit to the lifetime of oxygen-sensitive species suspected to be induced at critical sites in bacterial cells. Radiat. Res. 54: 171-180.

EWING, D. 1981. Hydroxyl radical damage in irradiated bacteria: a re-evaluation, p.269-275. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

FABRE, F. 1972. Relation between repair mechanisms and induced mitotic recombination after UV-irradiation in the yeast *Schizosaccharomyces pombe*. Effects of caffeine. Mol. Gen. Genet. 117: 153-166.

FARRINGTON, J.A., M. EBERT, E.J. LAND and K. FLETCHER. 1973. Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides. Biochim. Biophys. Acta 314: 372-381.

FEE, J.A. 1981. Is superoxide toxic and are superoxide dismutases essential for aerobic life?, p.205-239. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

FEE. J.A. 1982. Is superoxide important in oxygen poisoning? Trends Biochem. Sci. 7: 84-86.

FEINER, R.R. and R.F. HILL. 1963. Effect of basic dyes on host-cell reactivation of ultraviolet-irradiated phage. Nature 200: 291-293.

FELNER, J.M. & V.R. DOWELL. 1971. *Bacteroides* bacteremia. Am. J. Med. 50: 787-796.

FENN, W.O., R. GERSCHMAN, D.L. GILBERT, D.E. TERWILLIGER and F.V. COTHRAN. 1957. Mutagenic effects of high oxygen tensions on *Escherichia coli*. Proc. Natl. Acad. Sci. USA 43: 1027-1032.

FIELDEN, E.M., O. SAPORA and P.S. LOVEROCK. 1978. The application of rapid lysis techniques in radiobiology. III. The effect of radiosensitizers on the production of DNA damage and the time course of its repair. Radiat. Res. 75: 54-65.

FIIL, N., B. WILLUMSEN, J. FREISEN and K. VON MEYENBURG. 1977. Interaction of alleles of the *relA*, *relC* and *spoT* genes in *Escherichia coli:* analyses of the interconversion of GTP, ppGpp, and pppGpp. Mol. Gen. Genet. 150: 87-101.

FINEGOLD, S.M. 1970. The significance of the intestinal microflora. Infect. Dis. Rev. 1: 67-71.

FISHER, K.W. 1957. The role of the Krebs cycle in conjugation in *Escherichia coli* K12. J. Gen. Microbiol. 16: 120-135.

FLAKS, J.G., J. LICHTENSTEIN and S.S. COHEN. 1959. Virus-induced acquisition of metabolic function. II. Studies on the origin of the deoxycytidylate hydroxymethylase of bacteriophage-infected *E.coli*. J. Biol. Chem. 234: 1507-1511.

FLORY, J. and C.M. RADDING. 1982. Visualization of *recA* protein and its association with DNA: a priming effect of single-strand-binding protein. Cell 28: 747-756.

FLOWERS, R.S., S.E. MARTIN, D.G. BREWER and Z.J. ORDAL. 1977. Catalase and enumeration of stressed *Staphylococcus aureus* cells. Appl. Environ. Microbiol. 33: 1112-1117.

FOGLIANO, M. and P.F. SCHENDEL. 1981. Evidence for the inducibility of the *uvrB* operon. Nature 289: 196-198.

FONG, K. and R.C. BOCKRATH. 1979. Inhibition of deoxyribonucleic acid repair in *Escherichia coli* by caffeine and acriflavine after ultraviolet irradiation. J. Bacteriol. 139: 671-674.

FOX, R.A., E.M. FIELDEN and O. SAPORA. 1976. Yield of single-strand breaks in the DNA of *E.coli* 10 msec after irradiation. Int. J. Radiat. Biol. 29: 391-394.

FRANKE, A.E. & D.B. CLEWELL. 1981. Evidence for a chromosome-borne resistance transposon (Tn 916) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. 145: 494-502.

FREDETTE, V., C. PLANTE and A. ROY. 1967. Numerical data concerning the sensitivity of anaerobic bacteria to oxygen. J. Bacteriol. 94: 2012-2017.

FREESE, E.G., J. GERSON, H. TABER, H.J. RHAESE and E. FREESE. 1967. Inactivating DNA alterations induced by peroxides and peroxide-producing agents. Mutat. Res. 4: 517-531.

FREIFELDER, D. 1968. Physicochemical studies on X-ray inactivation of bacteriophage. Virology 36: 613-619.

FREY, H.E. and E.C. POLLARD. 1968. The action of gamma-ray-irradiated medium on bacteria: relation to the electron transport system. Radiat. Res. 36: 59-67.

FRIDOVICH, I. 1972. Superoxide radical and superoxide dismutase. Acc. Chem. Res. 5: 321-326.

FRIDOVICH, I. 1974. Superoxide dismutase. Adv. Enzymol. Relat. Areas Mol. Biol. 41: 35-97.

FRIDOVICH, I. 1975. Superoxide dismutase. Annu. Rev. Biochem. 44: 147-159.

FRIDOVICH, I. 1978. The biology of oxygen radicals. Nature 201: 875-880.

FRIDOVICH, I. 1981. The biology of superoxide and of superoxide dismutases - in brief, p.197-204. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

FRIEDBERG, E.C., T. BONURA, R. CONE, R. SIMMONS and C. ANDERSON. 1978. Base excision repair of DNA, p. 163-173. *In* P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), *DNA repair mechanisms*. Academic Press, New York.

FRIEDBERG, E.C., C.T.M. ANDERSON, T. BONURA, R. CONE, E.H. RADANY and R.J. REYNOLDS. 1981. Recent developments in the enzymology of excision repair of DNA. Prog. Nucl. Acid Res. Mol. Biol. 26: 197-216.

FUKS, Z. and K.C. SMITH. 1971. Effect of quinacrine on X-ray sensitivity and the repair of damaged DNA in *Escherichia coli* K12. Radiat. Res. 48: 63-73.

GALLANT, J.A. 1979. Stringent control in *Escherichia coli*. Annu. Rev. Genet. 13: 393-415.

GANESAN, A.K. 1974. Persistence of pyrimidine dimers during postreplication repair in ultraviolet light-irradiated *Escherichia coli* K12. J. Mol. Biol. 87: 103-119.

GANESAN, A.K. and K.C. SMITH. 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. II. Effect of *rec*$^-$ mutation on liquid holding recovery. J. Bacteriol. 96: 365-373.

GANESAN, A.K. and K.C. SMITH. 1969. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *uvr* genes on liquid holding recovery. J. Bacteriol. 97: 1129-1133.

GANESAN, A.K. and K.C. SMITH. 1972. Requirement for protein synthesis in *rec*-dependent repair of deoxyribonucleic acid in *Escherichia coli* after ultraviolet or X irradiation. J. Bacteriol 111: 575-585.

GEORGE.J., M. CASTELLAZZI and G. BUTTIN. 1975. Prophage induction and cell division in *E.coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. 140: 309-332.

GILLIES, N.E. and T. ALPER. 1959. Reduction in the lethal effects of radiations on *Escherichia coli* B by treatment with chloramphenicol. Nature 183: 237-238.

GLASS, T.L., W.M. HOLMES, P.B. HYLEMON and E.J. STELLWAG. 1979. Synthesis of guanosine tetra- and pentaphosphates by the obligately anaerobic bacterium *Bacteroides thetaiotaomicron* in response to molecular oxygen. J. Bacteriol. 137: 956-962.

GLICKMAN, B. 1974. The role of DNA polymerase I in pyrimidine dimer excision and repair replication in *Escherichia coli* K12 following ultraviolet irradiation. Biochim. Biophys. Acta 335: 115-122.

GOLDMARK. P.J. and S. LINN. 1970. An endonuclease activity from *Escherichia coli* absent from certain *rec*$^-$ strains. Proc. Natl. Acad. Sci. USA 67: 434-441.

GOTTESMAN, S. 1981. Genetic control of the SOS system in *E.coli*. Cell 23: 1-2.

GOTTESMAN, S. and D. ZIPSER. 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. 133: 844-851.

GOTTESMAN, S., E. HALPERN and P. TRISLER. 1981. Role of *sulA* and *sulB* in filamentation by *lon* mutants of *Escherichia coli* K-12. J. Bacteriol. 148: 265-273.

GOTTLEIB, S.F. 1971. Effects of hyperbaric oxygen on micro-organisms. Annu. Rev. Microbiol. 25: 111-152.

GREEN, A.R. and G. CURZON. 1968. Decrease of 5-hydroxy-tryptamine in the brain provoked by hydrocortisone and its prevention by allopurinol. Nature 220: 1095-1097.

GREGORY, E.M. and I. FRIDOVICH. 1973a. Oxygen toxicity and the superoxide dismutase. J. Bacteriol. 114: 1193-1197.

GREGORY, E.M. and I. FRIDOVICH. 1973b. Induction of superoxide dismutase by molecular oxygen. J. Bacteriol. 114: 543-548.

GREGORY, E.M., F.J. YOST, Jr. and I. FRIDOVICH. 1973. Superoxide dismutases of *Escherichia coli:* intracellular localization and functions. J. Bacteriol. 115: 987-991.

GREGORY, E.M. and I. FRIDOVICH. 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. 58: 57-62.

GREGORY, E.M., J.B. KOWALSKI and L.V. HOLDEMAN. 1977a. Production and some properties of catalase and superoxide dismutase from the anaerobe *Bacteroides distasonis.* J. Bacteriol. 129: 1298-1302.

GREGORY, E.M., B.J. VELTRI, D.L. WAGNER and T.D. WILKINS. 1977b. Carbohydrate repression of catalase synthesis in *Bacteroides fragilis.* J. Bacteriol. 129: 534-535.

GREGORY, E.M., W.E.C. MOORE and L.V. HOLDEMAN. 1978. Superoxide dismutase in anaerobes: survey. Appl. Environ. Microbiol. 35: 988-991.

GROSS, J.D., J. GRUNSTEIN and E.M. WITKIN. 1971. Inviability of *recA*$^-$ derivatives of DNA polymerase mutant of De Lucia and Cairns. J. Mol. Biol. 58: 631-634.

GROSSMAN, L., A. BRAUN, R. FELDBERG and I. MAHLER. 1975. Enzymatic repair of DNA. Annu. Rev. Biochem. 44: 19-43.

GUDAS, L.J. 1976. The induction of protein X in DNA repair and cell division mutants of *Escherichia coli*. J. Mol. Biol. 104: 567-587.

GUDAS, L.J. and A.B. PARDEE. 1975. Model for the regulation of *Escherichia coli* DNA repair functions. Proc. Natl. Acad. Sci. USA 72: 2330-2334.

GUDAS, L.J. and A.B. PARDEE. 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. 101: 459-477.

GUDAS, L.J. and D.W. MOUNT. 1977. Identification of the *recA(tif)* gene product of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 74: 5280-5284.

GUINEY, D.G. and C.E. DAVIS. 1975. Isolation of plasmid deoxyribonucleic acid from two strains of *Bacteroides*. J. Bacteriol. 124: 503-510.

HABER, F. and J. WEISS. 1934. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. R. Soc. London Ser. A 147: 332-351.

HALL, E.J. 1972. The effect of hypoxia on the repair of sublethal radiation damage in cultured mammalian cells. Radiat. Res. 49: 405-415.

HALL, J.D. and D.W. MOUNT. 1981. Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacteria and mammalian cells. Prog. Nucl. Acid Res. Mol. Biol. 25: 54-126.

HALLIWELL, B. 1982. Superoxide and superoxide-dependent formation of hydroxyl radicals are important in oxygen toxicity. Trends Biochem. Sci. 7: 270-272.

HAMKALO, B.A. and P.A. SWENSON. 1969. Effects of ultraviolet radiation on respiration and growth in radiation-resistant and radiation-sensitive strains of *Escherichia coli* B J. Bacteriol. 99: 815-823.

HANAWALT, P.C. 1966. The UV sensitivity of bacteria: its relation to the DNA replication cycle. Photochem. Photobiol. 5: 1-12.

HANAWALT, P.C. and R.B. SETLOW. 1960. Effect of monochromatic UV light on macromolecular synthesis in *Escherichia coli*. Biochim. Biophys. Acta 41: 283-294.

HANAWALT, P.C., P.K. COOPER, A.K. GANESAN and C.A. SMITH. 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. 48: 783-836.

HANAWALT, P.C., P.K. COOPER and C.A. SMITH. 1981. Repair replication schemes in bacteria and human cells. Prog. Nucl. Acid Res. Mol. Biol. 26: 181-196.

HANKE, M.E. and Y.J. KATZ. 1943. An electrolytic method for controlling oxidation-reduction potential and its application in the study of anaerobiosis. Arch. Biochem. Biophys. 2: 183-200.

HANSEN, M.T., M.L. PATO, S. MOLIN, N.P. FIIL and K. VON MEYENBURG. 1975. Simple downshift and resulting lack of correlation between ppGpp pool size and ribonucleic acid accumulation. J. Bacteriol. 122: 585-591.

HANSEN, S.L. and B.J. STEWART. 1978. Slide catalase: a reliable test for differentiation and presumptive identification of certain clinically significant anaerobes. Am. J. Clin. Pathol. 69: 36-40.

HARM, W. 1966. The role of host-cell repair in liquid-holding recovery of UV-irradiated *Escherichia coli*. Photochem. Photobiol. 5: 747-760.

HARM, W. 1967. Differential effects of acriflavine and caffeine on various ultraviolet-irradiated *Escherichia coli* strains and T1 phage. Mutat. Res. 4: 93-110.

HARM, W. 1970. Analysis of photoenzymatic repair of UV lesions in DNA by single light flashes. VIII. Inhibition of photoenzymatic repair of UV lesions in *Escherichia coli* by caffeine. Mutat. Res. 10: 319-333.

HARM, W. 1975. Kinetics of photoreactivation, p.89-101. *In* P.C. Hanawalt and R.B. Setlow (eds.), *Molecular mechanisms for repair of DNA*, Part A. Plenum Publishing Corp., New York.

HAROLD, F.M. and Z.Z. ZIPORIN. 1958. Synthesis of protein and of DNA in *Escherichia coli* irradiated with ultraviolet light. Biochim. Biophys. Acta 29: 439-440.

HARRIS, J.I., A.D. AUFFRET, F.D. NORTHROP and J.E. WALKER. 1980. Structural comparisons of superoxide dismutases. Eur. J. Biochem. 106: 297-303.

HARRISON, A.P., Jr., A.K. BRUCE and G.E. STAPLETON. 1958. Influence of X-irradiation on potassium retentivity by *Escherichia coli*. Proc. Soc. Exp. Biol. Med. 98: 740-743.

HARTMAN, P.S. and A. EISENSTARK. 1978. Synergistic killing of *Escherichia coli* by near-UV radiation and hydrogen peroxide: distinction between *recA*-repairable and *recA*-nonrepairable damage. J. Bacteriol. 133: 769-774.

HASAN, N. and E. NESTER. 1978. Purification and characterization of NADPH-dependent flavin reductase. An enzyme required for the activation of chorismate sythase in *Bacillus subtilis*. J. Biol. Chem. 253: 4987-4992.

HASSAN, H.M. and I. FRIDOVICH. 1977a. Enzymatic defences against the toxicity of oxygen and streptonigrin in *Escherichia coli*. J. Bacteriol. 129: 1574-1583.

HASSAN, H.M. and I. FRIDOVICH. 1977b. Physiological function of superoxide dismutase in glucose-limited chemostate-cultures of *Escherichia coli*. J. Bacteriol. 130: 805-811.

HASSAN, H.M. and I. FRIDOVICH. 1977c. Regulation of superoxide dismutase synthesis in *Escherichia coli:* glucose effect. J. Bacteriol. 132: 505-510.

HASSAN, H.M. and I. FRIDOVICH. 1978. Superoxide radical and the oxygen enhancement of the toxicity of paraquat in *Escherichia coli*. J. Biol. Chem. 253: 8143-8148.

HASSAN, H.M. and I. FRIDOVICH. 1979. Paraquat and *Escherichia coli*. Mechanism of production of extracellular superoxide radical. J. Biol. Chem. 254: 10846-10852.

HAUGAARD, N. 1968. Cellular mechanisms of oxygen toxicity. Physiol. Revs. 48: 311-373.

HELMSTETTER, C.E. and O. PIERRUCCI. 1968. Cell division during inhibition of deoxyribonucleic acid synthesis in *Escherichia coli*. J. Bacteriol. 95: 1627-1633.

HEWITT, J. and J.G. MORRIS. 1975. Superoxide dismutase in some obligately anaerobic bacteria. FEBS Lett. 50: 315-318.

HIGGINS, N.P., C.L. PEEBLES, A. SUGINO and N.R. COZZARELLI. 1978. Purification of subunits of *Escherichia coli* DNA gyrase and reconstitution of enzymatic activity. Proc. Natl. Acad. Sci. USA 75: 1773-1777.

HILL, M., B. DRASAR, V. ARIES, J. CROWTHER, G. HAWKSWORTH and R. WILLIAMS. 1971. Bacteria and the etiology of cancer of the large bowel. Lancet i:95: 95-100.

HODGSON, E.K. and I.FRIDOVICH. 1975. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide. Inactivation of the enzyme. Biochemistry 14: 5294-5299.

HOFSTAD, T. 1979. Serological responses to antigens of *Bacteroidaceae*. Microbiol. Rev. 43: 103-115.

HOLDEMAN, L.V. and W.E.C. Moore. 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.

HOLDEMAN. L.V. and W.E.C. MOORE (ed.). 1975. Anaerobe laboratory manual, 3rd ed. Anaerobe Laboratory, Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.

HOLLAENDER, A., G.E. STAPLETON and F. L. MARTIN. 1951. X-ray sensitivity of *E.coli* as modified by oxygen tension. Nature 167: 103-104.

HORII, T., T. OGAWA and H. OGAWA. 1980. Organization of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 77: 313-317.

HORII, T., T. OGAWA, T. NAKATANI, T. HASE, H. MATSUBARA and H. OGAWA. 1981a. Regulations of SOS functions: purification of *E.coli lexA* protein and determination of its specific site cleaved by the *recA* protein. Cell 27: 515-522.

HORII, T. T. OGAWA and H. OGAWA. 1981b. Nucleotide sequence of the *lexA* gene of *E.coli*. Cell. 23: 689-697.

HOSHINO, E., F. FRÖLANDER and J. CARLSSON. 1978. Oxygen and metabolism of *Peptostreptococcus anaerobius* VPI 4330-1. J. Gen. Microbiol. 107: 235-248.

HOSKINS, D.D., H.R., WHITELEY and B. MACKLER. 1962. The reduced diphosphopyridine nucleotide oxidase of *Streptococcus faecalis:* purification and properties. J. Biol. Chem. 237: 2647-2651.

HOWARD, A. and F.G. COWIE. 1978. Induced resistance in *Closterium:* indirect evidence for the induction of repair enzyme. Radiat. Res. 75: 607-616.

HOWARD-FLANDERS, P. 1958. Physical and chemical mechanisms in the injury of cells by ionizing radiations, p.553-586. *In* J.H. Laurence and C.A. Tobias (eds.), *Advances in Biological and Medical Physics*. Academic Press, New York.

HOWARD-FLANDERS, P. 1960. The effect of oxygen on the radiosensitivity of bacteriophage in the presence of sulfhydryl compounds. Nature 186: 485-487.

HOWARD-FLANDERS, P. and T. ALPER. 1957. The sensitivity of microorganisms to irradiation under controlled gas conditions. Radiat. Res. 7: 518-540.

HOWARD-FLANDERS, P. and D. MOORE. 1958. The time interval after pulsed irradiation within which injury to bacteria can be modified by dissolved oxygen. I. A search for an effect of oxygen 0,02 second after pulsed irradiation. Radiat. Res. 9: 422-437.

HOWARD-FLANDERS, P., E. SIMSON and L. THERIOT. 1964. The excision of thymine dimers from DNA, filament formation and sensitivity to ultraviolet light in *Escherichia coli* K-12. Mutat. Res. 1: 219-226.

HOWARD-FLANDERS, P. and R.P. BOYCE. 1966. DNA repair and genetic recombination; studies on mutants of *Escherichia coli* defective in these processes. Radiat. Res. 6 (Suppl): 156-184.

HOWARD-FLANDERS, P., R.P. BOYCE and L. THERIOT. 1966. Three loci in *Escherichia coli* K12 that control the excision of thymine dimers and certain other mutagen products from host or phage DNA. Genetics 53: 1119-1136.

HOWARD-FLANDERS, P. and L. THERIOT. 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics 53: 1137-1150.

HOWARD-FLANDERS, P., E. CASSUTO and S.C. WEST. 1981. Regulatory and enzymatic functions of *recA* protein in recombination and postreplication repair, p. 169-180. *In* E. Seeberg & K. Kleppe (eds.), *Chromosome damage and repair*. Plenum Publishing Corp., New York.

HOWE, W.E. and D.W. MOUNT. 1978. Analysis of cell division in single clones of the *Escherichia coli* K-12 *lexA* mutant. J. Bacteriol. 133: 1278-1281.

HOWE, W.E. and D.W. MOUNT. 1979. Distribution of cell lengths in cultures of a *lexA* mutant of *Escherichia coli* K-12. J. Bacteriol. 138: 273-274.

HUISMAN O., R. D'ARI and J. GEORGE. 1980a. Inducible *sfi* dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. 177: 629-636.

HUISMAN O., R. D'ARI and J. GEORGE. 1980b. Further characterization of *sfiA* and *sfiB* mutations in *Escherichia coli*. J. Bacteriol. 144: 185-191.

HUISMAN O. and R. D'ARI. 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature 290: 797-799.

HUISMAN, O. and R. D'ARI. 1983. Effect of suppressors of SOS-mediated filamentation on *sfiA* operon expression in *Escherichia coli*. J. Bacteriol. 153: 169-175.

HUISMAN, O., M. JACQUES, R. D'ARI and L. CARO. 1983. Role of the *sfiA*-dependent cell division regulation system in *Escherichia coli*. J. Bacteriol. 153: 1072-1074.

HUNGATE, R.E. 1969. A roll-tube method for the cultivation of strict anaerobes, p.117-132. *In* J.R. Norris and D.W. Ribbons (eds.), *Methods in Microbiology*, Vol.3B. Academic Press, London.

HUTCHINSON, F. 1961. Sulphydryl groups and the oxygen effect on irradiated dilute solutions of enzymes and nucleic acids. Radiat. Res. 14: 721-731.

HUTCHINSON, W.G. and M.A. MEDILL. 1954. The isolation of *Proteus* mutants and their use in recombination experiments. Bacteriol. Proc. 40-41.

INOUYE, M. 1971. Pleiotropic effect of the *recA* gene of *Escherichia coli:* uncoupling of cell division from deoxyribonucleic acid replication. J. Bacteriol. 106: 539-542.

INOUYE, M. and A.B. PARDEE. 1970. Changes of membrane proteins and their relation to DNA synthesis and cell division of *Escherichia coli*. J. Biol. Chem. 245: 5813-5819.

IRBE, R.M. and M. OISHI. 1980. Prophage induction in a permeabilized cell system: induction by deoxyribonucleases and the role of *recBC*-deoxyribonuclease. J. Bacteriol. 144: 1061-1067.

ISRAELI, E., A. KOHN and J. GITELMAN. 1975. The molecular nature of damage by oxygen to freeze-dried *Escherichia coli*. Cryobiology 12: 15-25.

ITAKURA, M. and E.W. HOLMES. 1979. Human amidophosphoribosyltransferase. An oxygen-sensitive iron-sulphur protein. J. Biol. Chem. 254: 333-338.

ITO, T. 1978. Cellular and subcellular mechanisms of photodynamic action: the $^1O_2$ hypothesis as a driving force in recent research. Photochem. Photobiol. 28: 493-508.

IYER, V.N. and W.D. RUPP. 1971. Usefulness of benzoylated naphthoylated DEAE-cellulose to distinguish and fractionate double-stranded DNA bearing different extends of single-stranded regions. Biochim. Biophys. Acta 228: 117-126.

JABLONSKI, E. and M. DELUCA. 1977. Purification and properties of the NADH and NADPH specific FMN oxidoreductases from *Beneckea harveyi*. Biochemistry 16: 2932-2936.

JACOBSON, L.A. and L.J. JACOBSON. 1980. Control of protein synthesis in *Escherichia coli*. Strain differences in control of translational initiation after energy source shiftdown. J. Bacteriol. 142: 888-898.

JOHANSEN, I. and P. HOWARD-FLANDERS. 1965. Macromolecular repair and free radical scavenging in the protection of bacteria against X rays. Radiat. Res. 24: 184-200.

JOHANSEN, I., I. GURVIN and W.D. RUPP. 1971. The formation of single-strand breaks in intracellular DNA by X-rays. Radiat. Res. 48: 599-612.

JOHANSEN, I., R. GULBRANDSEN and R. PETTERSEN. 1974. Effectiveness of oxygen in promoting X-ray-induced single-strand breaks in circular phage λ DNA and killing of radiation-sensitive mutants of *Escherichia coli*. Radiat. Res. 58: 384-397.

JOHANSEN, I., E. BOYE and T. BRUSTAD. 1975. Radiation induced strand breaks and time scale for repair of broken strands in superinfecting phage λ DNA in *E.coli* lysogenic for λ, p.267-274. *In* G.E. Adams, E.M. Fielden & B.D. Michael (eds.), *Fast processes in Radiation Chemistry and Biology*. The Institute of Physics and John Wiley & Sons, London.

JOHNSEN, K., S. MOLIN, O. KARLSTROM and O. MAALE. 1977. Control of protein synthesis in *Escherichia coli*. Analysis of an energy source shiftdown. J. Bacteriol. 131: 18-29.

JOHNSON, B.F. 1977. Fine structure mapping and properties of mutations suppressing the *lon* mutation in *Escherichia coli* K12 and B strains. Genet. Res. 30: 273-286.

JOHNSON, B.F. and J. GREENBERG. 1975. Mapping of *sul*, the suppressor of *lon* in *Escherichia coli*. J. Bacteriol. 122: 570-574.

JOHNSTON, M.A. and E.A. DELWICHE. 1965. Isolation and characterization of the cyanide-resistant and azide-resistant catalase of *Lactobacillus plantarum*. J. Bacteriol. 90: 352-356.

JONES, D.T. 1979. Genetic studies and physiological responses to ultraviolet radiation in the *Bacteroides fragilis* group. Ph.D. Thesis, Rhodes University, Republic of South Africa.

JONES, C.W., J.M. BRICE, V. WRIGHT and B.A.C. ACKRELL. 1973. Respiratory protection of a nitrogenase in *Azotobacter vinelandii*. FEBS Lett. 29: 77-81.

JONES, D.T., F.T. ROBB and D.R. WOODS. 1980. Effect of oxygen on *Bacteroides fragilis* survival after far-ultraviolet irradiation. J. Bacteriol. 144: 1179-1181.

JONES, D.T. and D.R. WOODS. 1981. Effect of oxygen on liquid holding recovery in *Bacteroides fragilis*. J. Bacteriol. 145: 1-7.

KACINSKI, B.M. and W.D. RUPP. 1981. *E.coli uvrB* protein binds to DNA in the presence of *uvrA* protein. Nature 294: 480-481.

KACINSKI, B.M., A. SANCAR and W.D. RUPP. 1981. A general approach for purifying proteins encoded by cloned genes without using a functional assay: isolation of the *UVRA* gene product from radiolabelled maxicells. Nucl. Acids Res 9: 4495-4508.

KAPP, D.S. and K.C. SMITH. 1970. Repair of radiation-induced damage in *Escherichia coli*. II. Effect of *rec* and *uvr* mutations on radiosensitivity, and repair of X-ray-induced single-strand breaks in deoxyribonucleic acid. J. Bacteriol. 103: 49-54.

KARI, C., Z. NAGY, P. KOVÁCS and F. HERNÁDI. 1971. Mechanism of the growth inhibitory effect of cysteine on *Escherichia coli*. J. Gen. Microbiol. 68: 349-356.

KATO, T. and S. KONDO. 1969. Genetic characters of X-ray sensitive mutants of *Escherichia coli* B. Jap. J. Genet. 44 (Suppl.1): 51-52.

KATO, T. and S. KONDO. 1970. Genetic and molecular characteristics of X-ray sensitive mutants of *Escherichia coli* defective in repair synthesis. J. Bacteriol. 104: 871-881.

KATO, T., R.H. ROTHMAN and A.J. CLARK. 1977. Analysis of the role of recombination and repair in mutagenesis of *Escherichia coli* by UV irradiation. Genetics 87: 1-18.

KAWAI, K., S. YASHIMA, Y. OKAMI and Y. SASAKI. 1971. Aerobic dissimilation of glucose by heterolactic bacteria. 1. Reduced pyridine nucleotide-oxidizing enzymes in *Leuconostoc mesenteroides*. J. Gen. Appl. Microbiol. 17: 51-62.

KEARNS, D.R. and A.U. KHAN. 1969. Sensitized photo-oxygenation reactions and the role of singlet oxygen. Photochem. Photobiol. 10: 193-210.

KEELE, B.B., Jr., J.M. McCORD and I. FRIDOVICH. 1970. Superoxide dismutase from *Escherichia coli* B. A new manganese-containing enzyme. J. Biol. Chem. 245: 6176-6181.

KELLER, R. and N. TRAUB. 1974. The characterization of *Bacteroides fragilis* bacteriophage recovered from animal sera: observations on the nature of *Bacteroides* phage carrier cultures. J. Gen. Virol. 24: 179-189.

KELLER, K.M. and E.C. POLLARD. 1977. Action of hydrogen peroxide on degradation of DNA after irradiation in *Escherichia coli*. Int. J. Radiat. Biol. 31: 407-413.

KELLOGG, E.W., III, and I. FRIDOVICH. 1977. Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide. J. Biol. Chem. 252: 6721-6728.

KELNER, A. 1953. Growth, respiration and nucleic acid synthesis in ultraviolet-irradiated and in photoreactivated *Escherichia coli*. J. Bacteriol. 65: 252-262.

KENYON, C.J. and G.C. WALKER. 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 77: 2819-2823.

KENYON, C.J. and G.C. WALKER. 1981. Expression of the *E.coli uvrA* gene is inducible. Nature 298: 808-810.

KESAVAN, P.C. 1973. Effect of caffeine and cysteine applied during post-irradiation hydration on an oxygen-independent component of damage in barley seeds. Radiat. Bot. 13: 355-359.

KESAVAN, P.C., S. TRASI and A. AHMAD. 1973. Modification of barley seed radiosensitivity by post-treatment with caffeine. 1. Effect of post-irradiation heat-shock and nature of hydration. Int. J. Radiat. Biol. 24: 581-587.

KESAVAN, P.C. and A. AHMAD. 1974. Influence of caffeine and cysteine on post-irradiation oxygen-dependent and independent components of damage in *Hordeum vulgare*. Experientia 30: 942.

KESAVAN, P.C. and S.M.J. AFZAL. 1975. Dependence of chemical radioprotection on the stability of radiation-induced oxygen-sensitive sites. Int. J. Radiat. Biol. 28: 495-499.

KESAVAN, P.C. and A. AHMAD. 1976. Modification of the radiosensitivity of barley seed by post-treatment with caffeine. III. Influence of dose of irradiation and concentration of caffeine. Int. J. Radiat. Biol. 29: 395-398.

KESAVAN, P.C. and N.J.F. DODD. 1976. Modification of radiation-induced oxic and anoxic damage by caffeine and potassium permanganate in dry and pre-soaked barley seeds. Int. J. Radiat. Biol. 30: 171-178.

KESAVAN, P.C., G.J. SHARMA and S.M.J. AFZAL. 1978. Differential modification of oxic and anoxic radiation damage by chemicals. 1. Simulation of the action of caffeine by certain inorganic radical scavengers. Radiat. Res. 75: 18-30.

KIMBALL, R.F., M.E. BOLING and S.W. PERDUE. 1977. Evidence that UV-inducible error-prone repair is absent in *Haemophilus influenzae* Rd, with a discussion of the relation to error-prone repair of alkylating-agent damage. Mutat. Res. 44: 183-196.

KOCH, C.J. and R.B. PAINTER. 1975. The effect of extreme hypoxia on the repair of DNA single-strand breaks in mammalian cells. Radiat. Res. 64: 256-269.

KOGOMA, T. and K.G. LARK. 1970. DNA replication in *Escherichia coli*: replication in absence of protein synthesis after replication inhibition. J. Mol. Biol. 52: 143-164.

KOGOMA, T. and K.G. LARK. 1975. Characterization of the replication of *Escherichia coli* DNA in the absence of protein synthesis: stable DNA replication. J. Mol. Biol. 94: 243-256.

KOGOMA, T. and M.J. CONNAUGHTON. 1978. Induced stable DNA replication as a possible SOS function. *7th Annual ICN-UCLA Symposium on Molecular and Cellular Biology.* Keystone, Colorado, Abstract 159.

KOGOMA, T., T.A. TORREY and M.J. CONNAUGHTON. 1979. Induction of UV-resistant DNA replication in *Escherichia coli*: induced stable DNA replication as an SOS function. Mol. Gen. Genet. 176: 1-9.

KONO, Y. and I. FRIDOVICH. 1982. Superoxide radicals inhibit catalase. J. Biol. Chem. 257: 5751-5754.

KROES, H.H., A. SCHEPMAN and A. RÖRSCH. 1963. The synthesis of ribosomal RNA in ultraviolet-irradiated *Escherichia coli*. Biochim. Biophys. Acta 76: 201-208.

LAEMMLI, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

LARK, K.G. 1972. Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli 15T⁻*. J. Mol. Biol. 64: 47-60.

LARK, K.G. and H. RENGER. 1969. Initiation of DNA replication in *15T⁻*. Chronological dissection of three physiological processes required for initiation. J. Mol. Biol. 42: 221-235.

LARK, K.G. and C.A. LARK. 1978. *recA*-Dependent DNA replication in the absence of protein synthesis: characteristics of a dominant lethal replication mutation, *dnaT*, and requirement for *recA⁺* function. Cold Spring Harbor Symp. Quant. Biol. 43: 537-549.

LASKEY, R.A. and A.D. MILLS. 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. 56: 335-341.

LAZZARINI, R.A., M. CASHEL and J. GALLANT. 1971. On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of *E.coli*. J. Biol. Chem. 246: 4381-4385.

LEHMAN, I.R. and J.R. CHIEN. 1973. Persistence of deoxyribonucleic acid polymerase I and its 5'-3' exonuclease activity in *polA* mutants of *Escherichia coli* K12. J. Biol. Chem. 248: 7717-7723.

LEHNERT, S. and H. MOROSON. 1971. X-ray-induced single-strand breaks in DNA of *E.coli* B/r: effect of irradiation and postirradiation conditions. Radiat. Res. 45: 299-310.

LEMBERG, R. and J.W. LEGGE. 1949. Hematin compounds and bile pigments, p. 415-444. Wiley Interscience, New York.

LIEBERMAN, R.P. and M. OISHI. 1974. The *recBC* deoxyribonuclease of *Escherichia coli* and characterization of the subunit proteins and reconstitution of the enzyme. Proc. Natl. Acad. Sci. USA 71: 4816-4820.

LINDAHL, T. 1979. DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision repair. Prog. Nucl. Acid Res. Mol. Biol. 22: 135-192.

LING, C.C., H.B. MICHAELS, E.R. EPP and E.C. PETERSON. 1978. Oxygen diffusion into mammalian cells following ultra-high dose rate irradiation and lifetime estimates of oxygen-sensitive species. Radiat. Res. 76: 522-532.

LIPSKY, R.H. and P.B. HYLEMON. 1980. Characterization of a NADH: flavin oxidoreductase induced by cholic acid in a 7α-dehydroxylating intestinal *Eubacterium* species. Biochim. Biophys. Acta 612: 328-336.

LITTLE, J.W. and P.C. HANAWALT. 1977. Induction of protein X in *Escherichia coli*. Mol. Gen. Genet. 150: 237-248.

LITTLE, J.W. and D.G. KLEID. 1977. *Escherichia coli* protein X is the *recA* gene product. J. Biol. Chem. 252: 6251-6252.

LITTLE, J.W. and J.E. HARPER. 1979. Identification of the *lexA* gene product of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA 76: 6147-6151.

LITTLE, J.W., S.H. EDMISTON, L.Z. PACELLI and D.W. MOUNT. 1980. Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA 77: 3225-3229.

LITTLE, J.W., D.W. MOUNT and C.R. YANISCH-PERRON. 1981. Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA 78: 4199-4203.

LITTLE J.W. and D.W. MOUNT. 1982. The SOS regulatory system of *Escherichia coli*. Cell 29: 11-22.

LIVINGSTON, D.M. and C.C. RICHARDSON. 1975. Deoxyribonucleic acid polymerase III of *Escherichia coli*. Characterization of associated exonuclease activities. J. Mol. Biol. 250: 470-478.

LOESCHE, W.J. 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18: 723-727.

LOW, E.I. and S.M. ZIMKUS. 1973. Reduced nicotinamide adenine dinucleotide oxidase activity and hydrogen peroxide formation of *Mycoplasma pneumoniae*. J. Bacteriol. 116: 346-354.

LUMSDEN, J. and D.O. HALL. 1975. Superoxide dismutase in photosynthetic organisms provides an evolutionary hypothesis. Nature 257: 670-672.

LUND, E. and N.O. KJELDGAARD. 1972. Metabolism of guanosine tetraphosphate in *Escherichia coli*. Eur. J. Biochem. 28: 316-326.

LYDERSEN, B.K. and E.C. POLLARD. 1975. The induction of λ-prophage by ionizing radiation. Radiat. Res. 64: 237-244.

LYNCH, R.E. and I. FRIDOVICH. 1978. Effects of superoxide on the erythrocyte membrane. J. Biol. Chem. 253: 1838-1845.

MAALØE, O. and P.C. HANAWALT. 1961. Thymine deficiency and the normal DNA replication cycle. J. Mol. Biol. 3: 144-155.

MACKAY, V. and S. LINN. 1976. Selective inhibition of the DNase activity of the *recBC* enzyme by the DNA binding protein from *Escherichia coli*. J. Biol. Chem. 251: 3716-3719.

MACKAY, D.B., T.A. STEITZ, I.T. WEBER, S.C. WEST and P. HOWARD-FLANDERS. 1980. Crystallization of monomeric *recA* protein. J. Biol. Chem. 255: 6662.

MALMSTRÖM, B.G. 1982. Enzymology of oxygen. Annu. Rev. Biochem. 51: 21-59.

MANCINI, C. and R.J. BEHME. 1977. Transfer of multiple antibiotic resistance from *Bacteroides fragilis* to *Escherichia coli*. J. Infect. Dis. 136: 597-600.

MARKHAM, B.E., J.W. LITTLE and D.W. MOUNT. 1981. Nucleotide sequence of the *lexA* gene of *Escherichia coli* K-12. Nucl. Acids Res. 9: 4149-4161.

MARKLUND, S. and G. MARKLUND. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47: 469-474.

MARSDEN, H.S., E.C. POLLARD, W. GINOZA and E.P. RANDALL. 1974. Involvement of *recA* and *exr* genes in the *in vivo* inhibition of the *recBC* nuclease. J. Bacteriol. 118: 465-470.

MARTIN, W.J. 1971. Practical method for isolation of anaerobic bacteria in the clinical laboratory. Appl. Microbiol. 22: 1168-1171.

MARTIN, S.E., R.S. FLOWERS and Z.J. ORDAL. 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. 32: 731-734.

MASSIE, H., H. SAMIS and M. BAIRD. 1972. The kinetics of degradation of DNA and RNA by H_2O_2. Biochim. Biophys. Acta 272: 539-548.

MASUDA, T., J. OVADIA and L.I. GROSSWEINER. 1971. The pulse radiolysis and inactivation of trypsin. Int. J. Radiat. Biol. 20: 447-459.

MAYS, T.D., F.L. MACRINA, R.A. WELCH and C. J. SMITH. 1981. Unusual conjugal transfer of antibiotic resistance in *Bacteroides* : non-involvement of plasmids, p. 631. *In* S.B. Levy, R.C. Clowes and E.L. Koenig (eds.), *Molecular Biology, pathogenicity, and ecology of bacterial plasmids.* Plenum Publishing Corp., New York.

MAYS, D.T., C.J. SMITH, R.A. WELCH, C. DELFINI and F.L. MACRINA. 1982. Novel antibiotic resistance transfer in *Bacteroides*. Antimicrob. Agents Chemother. 21: 110-118.

McCORD, J.M. 1979. Superoxide, superoxide dismutase and oxygen toxicity, p. 109-124. *In* E. Hodgson, J.R. Bend, and R.M. Philpot (eds.), *Reviews in biochemical toxicology*. Elsevier/North-Holland Publishing Co., New York.

McCORD, J.M. and I. FRIDOVICH. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049-6055.

McCORD, J.M., B.B. KEELE and I. FRIDOVICH. 1971. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. USA 68: 1024-1027.

McENTEE, K. 1976. Specialyzed transduction of *recA* by bacteriophage lambda. Virology 70: 221-222.

McENTEE, K. and W. EPSTEIN. 1977. Isolation and characterization of specialized transducing bacteriophages for the *recA* gene of *Escherichia coli*. Virology 77: 306-318.

McENTEE, K. and G.M. WEINSTOCK. 1981. *tif*-1 mutation alters polynucleotide recognition by the *recA* protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 78: 6061-6065.

McENTEE, K., G.M. WEINSTOCK and I.R. LEHMAN. 1981. Binding of the *recA* protein of *Escherichia coli* to single- and double-stranded DNA. J. Biol. Chem. 256: 8835-8844.

McPARTLAND, A., L. GREEN and H. ECHOLS. 1980. Control of *recA* gene RNA in *E.coli*: regulatory and signal genes. Cell 20: 731-737.

MERRICK, T.P. and A.K. BRUCE. 1965. Radiation response of potassium efflux in *Micrococcus radiodurans* and *Sarcina lutea*. Radiat. Res. 24: 612-618.

MEYER, R.R., J. GLASSBERG and A. KORNBERG. 1979. An *E.coli* mutant defective in single-strand binding protein is defective in DNA replication. Proc. Natl. Acad. Sci. USA 76: 1702-1705.

MEYER, R.R., J. GLASSBERG, J.V. SCOTT and A. KORNBERG. 1980. A temperature-sensitive single-stranded DNA binding protein from *E.coli*. J. Biol. Chem. 255: 2897-2901.

MICHAEL, B.D., H.A. HARROP and K.D. HELD. 1981. Time-scale and mechanisms of the oxygen effect in irradiated bacteria, p.285-292. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

MICHAELIS, L. 1929. Oxidation-reduction systems of biological significance. VI. The mechanism of the catalytic effect of iron on the oxidation of cysteine. J. Biol. Chem. 84: 777-787.

MICHAELS, B.D., G.E. ADAMS, H.B. HEWITT, W.E.G. JONES and M.E. WATTS. 1973. A post-effect of oxygen in irradiated bacteria: a submillisecond fast mixing study. Radiat. Res. 54: 239-251.

MICHAELS, H.B., E.R. EPP and C.C. LING. 1981. Exploring the time scale of radiosensitization, p.335-347. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

MICHALKE, H. and H. BREMER. 1969. RNA synthesis in *Escherichia coli* after irradiation with ultraviolet light. J. Mol. Biol. 41: 1-23.

MICHEL, R.E.J. 1975. Involvement of hydroxyl radicals in the release by ionizing radiation of a cell surface nuclease from *Micrococcus radiodurans*. Radiat. Res. 64: 321-330.

MIKI, T., Y. EBINA, F. KISHI and A. NAKAZAWA. 1981. Organization of the *lexA* gene of *Escherichia coli* and nucleotide sequence of the regulatory region. Nucl. Acids Res. 9: 529-543.

MILLER, H.I. 1981. Multilevel regulation of bacteriophage λ lysogeny by the *E.coli himA* gene. Cell 25: 269-276.

MILLER, H.I. and D.I. FRIEDMAN. 1980. An *E.coli* gene product required for λ site-specific recombination. Cell 20: 711-719.

MILLER, H.I., M. KIRK and H. ECHOLS. 1981. SOS induction and autoregulation of the *himA* gene for site-specific recombination in *E.coli*. Proc. Natl. Acad. Sci. USA 78: 6754-6758.

MILLER, H.I. and H.A. NASH. 1981. Direct role of the *himA* gene product in phage λ integration. Nature 290: 523-526.

MISRA, H.P. and I. FRIDOVICH. 1976. Superoxide dismutase and the oxygen enhancement of radiation lethality. Arch. Biochem. Biophys. 176: 577-581.

MISRA, H.P. and I. FRIDOVICH. 1977. Purification and properties of superoxide dismutase from a red alga *Porphyridium cruentum*. J. Biol. Chem. 252: 6421-6423.

MIZUSAWA, S. and S. GOTTESMAN. 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of *sulA* protein. Proc. Natl. Acad. Sci. USA 80: 358-362.

MIZUSHIMA, S. and K. KITAHARA. 1962. Purification and properties of DPNH peroxidase in *Lactobacillus casei*. J. Gen. Appl. Microbiol. 8: 56-62.

MONK, M. and J. GROSS. 1971. Induction of prophage λ in a mutant of *E.coli* K12 defective in initiation of DNA replication at high temperatures. Mol. Gen. Genet. 110: 299-306.

MONK, M., M. PEACEY and J.D. GROSS. 1971. Repair of damage induced by ultraviolet light in DNA polymerase-defective *Escherichia coli* cells. J. Mol. Biol. 58: 623-630.

MONK, M. and J. KINROSS. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. 109: 971-978.

MOODIE, H.L. and D.R. WOODS. 1973. Anaerobic R factor transfer in *Escherichia coli*. J. Gen. Microbiol. 76: 437-440.

MOODY, C.S. and H.M. HASSAN. 1982. Mutagenicity of oxygen free radicals. Proc. Natl. Acad. Sci. USA 79: 2855-2859.

MOORE, W.E.C. 1966. Techniques for routine culture of fastidious anaerobes. Int. J. Syst. Bacteriol. 16: 173-190.

MOORE, W.E.C. and L.V. HOLDEMAN. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27: 961-979.

MOORE, P.D., K.K. BOSE, S.D. RABKIN and B.S. STRAUSS. 1981. Sites of termination of *in vitro* DNA synthesis on ultraviolet and N-acetylaminofluorene treated ØX174 templates by prokaryotic and eukaryotic DNA polymerases. Proc. Natl. Acad. Sci. USA 78: 110-114.

MORAND, P., M. BLANCO and R. DEVORET. 1977. Characterization of *lexB* mutations in *Escherichia coli* K12. J. Bacteriol. 131: 572-582.

MORIMYO, M. 1982. Anaerobic incubation enhances the colony formation of a *polA recB* strain of *Escherichia coli* K12. J. Bacteriol. 152: 208-214.

MORIMYO, M., Z. HORII and K. SUZUKI. 1968. Appearance of low molecular weight DNA in a *rec*$^-$ mutant of *Escherichia coli* K-12 irradiated with X-rays. J. Radiat. Res. 9: 19-25.

MORIMYO, M. and Y. SHIMAZU. 1976. Evidence that the gene *uvrB* is indispensable for polymerase I deficient strain of *Escherichia coli* K-12. Mol. Gen. Genet. 147: 243-250.

MOROZOV, I.I. and M.N. MYASNIK. 1970. The effect of chloramphenicol on survival of ultraviolet-irradiated cells of *Escherichia coli* B_{s-1}. Stud. Biophys. 20: 43.

MORRIS, J.G. 1970. The biochemical basis of oxygen toxicity. J. Gen. Microbiol. 60: iii.

MORRIS, J.G. 1975. The physiology of obligate anaerobiosis. Adv. Microbiol. Physiol. 12: 169-246.

MORRIS, J.G. 1976. Oxygen and the obligate anaerobe. J. Appl. Bacteriol. 40: 229-244.

MORTELMANS, K.E. and B.A.D. STOCKER. 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. 167: 317-328.

MOSBAUGH, D.W. and S. LINN. 1980. Further characterization of human fibroblasts apurinic/apyrimidinic DNA endonucleases. J. Biol. Chem. 255: 11743-11752.

MOSS, S.H. and D.J.G. DAVIES. 1974. Interrelationship of repair mechanisms in ultraviolet-irradiated *Escherichia coli*. J. Bacteriol. 120: 15-23.

MOSSIE, K.G., D.T. JONES, F.T. ROBB and D.R. WOODS. 1979. Characterization and mode of action of a bacteriocin produced by a *Bacteroides fragilis* strain. Antimicrob. Agents and Chemother. 16: 724-730.

MYERS, D.M. 1970. Some aspects of radiation effects on cell membranes, p. 219-234. *In* J.H. Lawrence and J.W. Gofman (eds.), *Advances in Biological and Medical Physics*, Vol. 13. Academic Press, New York/London.

NADKARNI, S. and P.C. KESAVAN. 1975. Modification of barley seed radiosensitivity by post-treatment with caffeine, II. Kinetics of decay of caffeine-reactive oxygen-sensitive sites. Int. J. Radiat. Biol. 27: 569-576.

NATHAN, C.F. 1982. Secretion of oxygen intermediates: role in effector functions of activated macrophages. Federation Proceedings 41: 2206-2211.

NEUHARD, J. and E. THOMASSEN. 1971. Turnover of the deoxyribonucleoside triphosphates in *Escherichia coli* 15T during thymine starvation. Eur. J. Biochem. 20: 36-43.

NIERLICH, D.P. 1978. Regulation of bacterial growth, RNA, and protein synthesis. Annu. Rev. Microbiol. 32: 393-432.

NORMAN. A. and S. BERGMAN. 1960. The action of intestinal microorganisms on bile acids. Acta. Chem. Scand. 14: 1781-1785.

NOTANI, N.K. and J.K. SETLOW. 1980. Inducible repair system in *Haemophilus influenzae* unaccompanied by mutation. J. Bacteriol. 143: 516-519.

O'BRIEN, R.W. and J.G. MORRIS. 1971. Oxygen and the growth and metabolism of *Clostridium acetobutylicum*. J. Gen. Microbiol. 68: 307-318.

ODAJIMA, T. and I. YAMAZAKI. 1970. Myeloperoxidase of the leukocyte of normal blood. I. Reaction of myeloperoxidase with hydrogen peroxide. Biochim. Biophys. Acta 206: 71-77.

ODAJIMA, T. and I. YAMAZAKI. 1972. Myeloperoxidase of the leukocyte of normal blood. III. The reaction of ferric myeloperoxidase with superoxide anion. Biochim. Biophys. Acta 284: 355-359.

O'FARRELL, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007-4021.

OGAWA, H., K. SHIMADA and J. TOMIZAWA. 1968. Studies on radiation-sensitive mutants of *E.coli*. I. Mutants defective in the repair synthesis. Mol. Gen. Genet. 101: 227-244.

OGAWA, T., H. WABIKO, T. TSURIMOTO, T. HORII, H. MASUKATA and H. OGAWA. 1979. Characterization of purified *recA* protein and the regulation of its synthesis *in vivo*. Cold Spring Harbor Symp. Quant. Biol. 43: 909-915.

OISHI, M. 1969. An ATP-dependent deoxyribonuclease from *Escherichia coli* with a possible role in genetic recombination. Proc. Natl. Acad. Sci. USA 64: 1294-1299.

OISHI, M. and C.L. SMITH. 1978. Inactivation of phage repressor in a permeable cell system: role of *recBC* DNase in induction. Proc. Natl. Acad. Sci. USA 75: 3569-3573.

OISHI, M., C.L. SMITH and B. FRIEFELD. 1978. Molecular events and molecules that lead to induction of prophage and SOS functions. Cold Spring Harbor Symp. Quant. Biol. 43: 897-907.

OISHI, M., R.M. IRBE and L.M.E. MORIN. 1981. Molecular mechanism for the induction of "SOS" functions. Prog. Nucl. Acid Res. Mol. Biol. 26: 281-301.

OKAGAKI, H. 1960. Effect of chloramphenicol on the survival of *Escherichia coli* irradiated with ultraviolet light. J. Bacteriol. 79: 277-291.

OKAZAKI, R., M. ARISAWA and A. SUGINO. 1971. Slow joining of newly replicated DNA chains in DNA polymerase I-deficient *Escherichia coli* mutants. Proc. Natl. Acad. Sci. USA 68: 2954-2957.

OLSEN, J. and L. DAVIS. 1976. Oxidation of dithiothreitol by peroxidases and oxygen. Biochim. Biophys. Acta 445: 324-329.

ONDERDONK, A.B., J. JOHNSTON, J.W. MAYHEW and S.L. GORBACH. 1976. Effect of dissolved oxygen and Eh on *Bacteroides fragilis* during continuous culture. Appl. Environ. Microbiol. 31: 168-172.

ONDERDONK, A.B., D.L. KASPER, R.L. CISNEROS and J.G. BARTLETT. 1977. The capsular polysaccharide of *B.fragilis* as a virulence factor: comparison of pathogenic potential of encapsulated and unencapsulated strains. J. Infect. Dis. 136: 32-39.

PASTAN, I. and S. ADHYA. 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. 40: 527-551.

PEAK, M.J., J.G. PEAK and R.B. WEBB. 1973. Inactivation of transforming DNA by ultraviolet light. III. Further observations on the effects of 365 nm radiation. Mutat. Res. 20: 143-148.

PEAK, J.G., C.S. FOOTE and M.J. PEAK. 1981. Protection by Dabco against inactivation of transforming DNA by near-ultraviolet light: action spectra and implications for involvement of singlet oxygen. Photochem. Photobiol. 34: 45-49.

PEDERSEN, S., P.L. BLOCH, S. REEH and F.C. NEIDHARDT. 1978. Patterns of protein synthesis in *E.coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell. 14: 179-190.

PERRY, K.L. and G.C. WALKER. 1982. Identification of plasmid (pKM101)- coded proteins involved in mutagenesis and UV resistance. Nature 300: 278-281.

PETRANOVIĆ, M., E. SALAJ-ŠMIC, D. PETRANOVIĆ and Ž. TRGOVČEVIĆ. 1979. Inactivation of prophage in UV-irradiated *Escherichia coli*: dependence on *recA* gene activity. J. Bacteriol. 140: 848-851.

PHIZICKY, E.M. and J.W. ROBERTS. 1981. Induction of SOS functions: regulation of proteolytic activity of *E.coli recA* protein by interaction with DNA and nucleoside triphosphate. Cell 25: 259-267.

POLLARD, E.C. and P.K. WELLER. 1967. Chain scission of ribonucleic acid and deoxyribonucleic acid by ionizing radiation and hydrogen peroxide *in vitro* and in *Escherichia coli* cells. Radiat. Res. 32: 417-440.

POLLARD, E.C. and W. SNIPES. 1977. Absence of two radiation-induced repair processes in a marine pseudomonad. Photochem. Photobiol. 26: 545-548.

POLLARD, E.C., D.J. FLUKE and D. KAZANIS. 1981. Comparative induction studies. Prog. Nucl. Acid Res. Mol. Biol. 26: 315-322.

POWERS, E.L., R.B. WEBB and C.F. EHRET. 1960. Storage, transfer and utilization of energy from X-rays in dry bacterial spores. Radiat. Res. Suppl. 2: 94-121.

PRIVITERA, G.A., A. DUBLANCHET and M. SEBALD. 1979a. Transfer of multiple antibiotic resistance between subspecies of *Bacteroides fragilis*. J. Infect. Dis. 139: 97-101.

PRIVITERA, G., M. SEBALD and F. FAYOLLE. 1979b. Common regulatory mechanism of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*. Nature 278: 657-659.

QUILLARDET, P., O. HUISMAN, R. D'ARI and M. HOFNUNG. 1982. SOS chromotest, a direct assay of induction of an SOS function in *E.coli* K12 to measure genotoxicity. Proc. Natl. Acad. Sci. USA 79: 5971-5975.

QUINTO, G. 1966. Amino acid and vitamin requirements of several *Bacteroides* strains. Appl. Microbiol. 14: 1022-1026.

RADMAN, M. 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355-367. *In* P.C. Hanawalt and R.B. Setlow (eds.), *Molecular mechanisms for repair of DNA*. Plenum Publishing Corp., New York.

RADMAN, M., L. CORDONE, D. KRSMANOVIĆ-SIMIC and M. ERRERA. 1970. Complementary action of recombination and excision in the repair of ultraviolet irradiation damage to DNA. J. Mol. Biol. 49: 203-212.

RAJAGOPALAN, K.V. and P. HANDLÉR. 1964. Hepatic aldehyde oxidase. II. Differential inhibition of electron transfer to various electron acceptors. J. Biol. Chem. 239: 2022-2026.

RASHTCHIAN, A. and S.J. BOOTH. 1981. Stability in *Escherichia coli* of an antibiotic resistance plasmid from *Bacteroides fragilis*. J. Bacteriol. 146: 212-227.

RASHTCHIAN, A., G.R. DUBES and S.J. BOOTH. 1982. Tetracycline-inducible transfer of tetracycline resistance in *Bacteroides fragilis* in the absence of detectable plasmid DNA. J. Bacteriol. 150: 141-147.

RAYMAN, M.K., B. ARIS and H.B. EL DEREA. 1978. The effect of compounds which degrade hydrogen peroxide on the enumeration of heat-stressed cells of *Salmonella senftenberg*. Can. J. Microbiol. 24: 883-885.

REED, R.R., R.A. YOUNG, J.A. STEITZ, N.D.F. GRINDLEY and M.S. GUYER. 1979. Transposition of the *Escherichia coli* insertion element λ generates a five-base-pair repeat. Proc. Natl. Acad. Sci. USA 76: 4882-4886.

RESNICK, J. and R. SUSSMAN. 1982. Cobalt: an effector of *E.coli recA* protein activity. Nucl. Acids Res. 10: 5239-5253.

RHAESE, H.J. and E. FREESE. 1968. Chemical analysis of DNA alterations. I. Base liberation and backbone breakage of DNA and oligodeoxyadenylic acid induced by hydrogen peroxide and hydroxylamine. Biochim. Biophys. Acta 155: 476-490.

RICHTER, D. 1980. *In vitro* degradation of guanosine 3'5'-bis (diphosphate) (ppGpp) by the *spoT* gene product (ppGppase) from auxotrophic strains of *Escherichia coli:* effects of various antibiotics and drugs. Arch. Microbiol. 124: 229-232.

ROBERTS, J.W., C.W. ROBERTS and N.L. CRAIG. 1978. *Escherichia coli recA* gene product inactivates phage λ repressor. Proc. Natl. Acad. Sci. USA 75: 4714-4718.

ROBERTS, J.W., C.W. ROBERTS, N.L. CRAIG and E. PHIZICKY. 1979. Activity of the *Escherichia coli recA* gene product. Cold Spring Harbor Symp. Quant. Biol. 43: 917-920.

ROLFE, R.D., D.J. HENTGES, J.T. BARRETT and B.J. CAMPBELL. 1977. Oxygen tolerance of human intestinal anaerobes. Am. J. Clin. Nutr. 30: 1762-1769.

ROLFE, R.D., D.J. HENTGES, B.J. CAMPBELL and J.T. BARRETT. 1978. Factors related to the oxygen tolerance of anaerobic bacteria. Appl. Environ. Microbiol. 36: 306-313.

ROTHMAN, R.H. 1980. Dimer excision in *Escherichia coli* in the presence of caffeine. J. Bacteriol. 143: 520-524.

ROTHMAN, R.H., L.J. MARGOSSIAN and A.J. CLARK. 1979. W-reactivation of phage lambda in *recF*, *recL*, *uvrA*, and *uvrB* mutants of *E.coli* K-12. Mol. Gen. Genet. 169: 279-287.

ROULLAND-DUSSOIX, D. 1967. Dégradation par la cellule hôte du DNA du bactériophage lambda irradié par le rayonnement ultraviolet. Mutat. Res. 4: 241-252.

RUDÉ, J.M. & C.O. DOUDNEY. 1973. Relation between survival and deoxyribonucleic acid replication in ultraviolet-irradiated resistant and sensitive strains of *Escherichia coli* B/r. J. Bacteriol. 113: 1161-1169.

RUPERT, C.S. 1975. Enzymatic photoreactivation: overview, p. 73-87. *In* P. Hanawalt and R.B. Setlow (eds.), *Molecular mechanisms for repair of DNA*, Part A. Plenum Publishing Corp., New York.

RUPERT, C.S. and W. HARM. 1966. Reactivation after photobiological damage. Adv. in Radiat. Biol. 2: 1-81.

RUPP, W.D. and P. HOWARD-FLANDERS. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. 31: 291-304.

RUPP, W.D., C.E. WILDE III, D.L. RENO and P. HOWARD-FLANDERS. 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J. Mol. Biol. 61: 25-44.

SALLES, B. and C. PAOLETTI. 1983. Control of UV induction of *recA* protein. Proc. Natl. Acad. Sci. USA 80: 65-69.

SALYERS, A.A., J.R. VERCELLOTTI, S.E.H. WEST and T.D. WILKINS. 1977. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. Appl. Environ. Microbiol. 33: 319-322.

SALYERS, A.A. and S.F. KOTARSKI. 1980. Induction of chondroitin sulfate lyase activity in *Bacteroides thetaiotaomicron*. J. Bacteriol. 143: 781-788.

SAMAH, O.A. and J.W.T. WIMPENNY. 1982. Some effects of oxygen on the physiology of *Selenomonas ruminantium* WPL 151/1 grown in continuous culture. J. Gen. Microbiol. 128: 355-360.

SAMUNI, A., M. CHEVION, Y.S. HALPERN, Y.A. ILAN and G. CZAPSKI. 1978. Radiation-induced damage in T4 bacteriophage: the effect of superoxide radicals and molecular oxygen. Radiat. Res. 75: 489-496.

SANCAR, A., A.M. HACK and W.D. RUPP. 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. 137: 692-693.

SANCAR, A. & W.D. RUPP. 1979. Physical map of the *recA* gene. Proc. Natl. Acad. Sci. USA 76: 3144-3148.

SANCAR, A., C. STACHELEK, W. KONIGSBERG and W.D. RUPP. 1980. Sequences of the *recA* gene and protein. Proc. Natl. Acad. Sci. USA 77: 2611-2615.

SANCAR, A., N.D. CLARKE, J. GRISWOLD, W.J. KENNEDY and W.D. RUPP. 1981a. Identification of the *uvrB* gene product. J. Mol. Biol. 148: 63-76.

SANCAR, A., R.P. WHARTON, S. SELTZER, B.M. KACINSKI, N.D. CLARKE and W.D. RUPP. 1981b. Identification of the *uvrA* gene product. J. Mol. Biol. 148: 45-62.

SANCAR, G.B., A. SANCAR, J.W. LITTLE and W.D. RUPP. 1982. The *uvrB* gene of *Escherichia coli* has both *lexA*-repressed and *lexA*-independent promoters. Cell 28: 523-530.

SAPORA, O., E.E. FIELDEN and P.S. LOVEROCK. 1975. The application of rapid lysis techniques in radiobiology. I. The effect of oxygen and radiosensitizers on DNA strand break production and repair in *E.coli* B/r. Radiat. Res. 64: 431-442.

SAPORA, O., E.M. FIELDEN and P.S. LOVEROCK. 1977a. A comparative study of the effect of two types of radiosensitizers on the survival of several *E.coli* B and K12 mutants. Radiat. Res. 69: 293-305.

SAPORA, O., E.M. FIELDEN and P.S. LOVEROCK. 1977b. The application of rapid lysis techniques in radiobiology. II. The time course of the repair of DNA fixed damage, and single-strand breaks following irradiation of *E.coli* mutants. Radiat. Res. 72: 308-316.

SATTA, G., L.J. GUDAS and A.B. PARDEE. 1979. Degradation of *Escherichia coli* DNA : evidence for limitation *in vivo* by protein X, the *recA* gene product. Mol. Gen. Genet. 168: 69-80.

SAUERBIER, W. 1964. Inhibition of host cell reactivation in phage T1 by caffeine. Biochem. Biophys. Res. Commun. 14: 340-346.

SAUNDERS, B.C. 1973. Peroxidases and catalases, p.988-1021. *In* G.L. Eichhorn (ed.), *Inorganic Biochemistry*, Vol.2. Elsevier, Amsterdam.

SCHENDEL, P.F., M. FOGLIANO and L. D. STRAUSSBAUGH. 1982. Regulation of the *Escherichia coli* K-12 *uvrB* operon. J. Bacteriol. 150: 676-685.

SCHENLEY, R.L., W.D. FISHER and P.A. SWENSON. 1976a. Pyrimidine dimer excision in surviving and nonsurviving cells of ultraviolet-irradiated cultures of *Escherichia coli*. J. Bacteriol. 126: 985-989.

SCHENLEY, R.L., W.D. FISHER, P.A. SWENSON and G.G. KHACHATOURIANS. 1976b. Centrifugal separation of irradiated cultures of *Escherichia coli* cells into viable and nonviable populations. J. Bacteriol. 126: 977-984.

SCHONBAUM, G.R. and B. CHANCE. 1976. Catalase, p. 363-408. *In* P. Boyer (ed.), *The enzymes*, Vol.13. Academic Press Inc., New York.

SCHUMANN, J.P., D.T. JONES and D.R. WOODS. 1982. UV light induction of proteins in *Bacteroides fragilis* under anaerobic conditions. J. Bacteriol. 151: 44-47.

SCHUSTER, H., D. BEYERSMANN, M. MIKOLAJCZKY and M. SCHLICHT. 1973. Prophage induction by high temperature in thermosensitive *dna* mutants lysogenic for bacteriophage lambda. J. Virology 11: 879-885.

SEDGWICK, S. and B.A. BRIDGES. 1974. Requirement for either DNA polymerase I or DNA polymerase III in post-replication repair in excision proficient *Escherichia coli*. Nature 249: 348-249.

SEDGWICK, S.G., A. LEVINE and A. BAILONE. 1978. Induction of *recA*$^{+}$-protein synthesis in *Escherichia coli*. Mol. Gen. Genet. 160: 267-276.

SEDLIAKOVÁ, M., J. PRACHAR and F. MASEK. 1977. Dependence of DNA dark repair on protein synthesis in *Escherichia coli*. Mol. Gen. Genet. 153: 23-27.

SEDLIAKOVÁ, M. and V. SLEZARIKOVÁ. 1977. Involvement of UV-induced protein in the reinitiation of DNA replication in UV-irradiated *Escherichia coli*. Folia microbiologica 22: 313-316.

SEEBERG, E. 1978. Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated *uvrA*$^+$ and *uvrB*$^+$/*uvrC*$^+$ gene products. Proc. Natl. Acad. Sci. USA 75: 2569-2573.

SEEBERG, E. 1981 Multiprotein interactions in strand cleavage of DNA damaged by UV and chemicals. Prog. Nucl. Acid Res. Mol. Biol. 26: 217-226.

SEEBERG, E. and W.D. RUPP. 1975. Effect of mutations in *lig* and *polA* on UV-induced strand cutting in *uvrC* strain of *E.coli*, p.439-441. *In* P.C. Hanawalt and R.B. Setlow (eds.), *Molecular mechanisms for the repair of DNA*. Plenum Publishing Corp., New York.

SEEBERG, E., J. NISSEN-MEYER and P. STRIKE. 1976. Incision of ultraviolet-irradiated DNA by extracts of *E.coli* requires three different gene products. Nature 263: 524-526.

SEEBERG, E. and P. STRIKE. 1976. Excision repair of ultraviolet-irradiated deoxyribonucleic acid in plasmolyzed cells of *Escherichia coli*. J. Bacteriol. 125: 787-795.

SETLOW, R.B. 1967. Steps in the repair of DNA: fact and fancy, p. 1-16. Brookhaven Symposium in Biology, No. 20. Federal Scientific and Technical Information Clearing House, Springfield, Va., for the Brookhaven National Laboratory.

SETLOW, R.B., P.A. SWENSON and W.L. CARRIER. 1963. Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. Science 142: 1464-1466.

SETLOW, R.B. and W.L. CARRIER. 1964. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Natl. Acad. Sci. USA 51: 226-231.

SETLOW, R.B. and W.L. CARRIER. 1966. Pyrimidine dimers in ultraviolet irradiated DNA. J. Mol. Biol. 17: 237-254.

SETLOW, R.B. and W.L. CARRIER. 1968. The excision of pyrimidine dimers *in vivo* and *in vitro*, p.134-141. *In* W.J. Peacock and R.D. Brock (eds.), *Replication and recombination of genetic material.* Australian Academy of Science, Canberra.

SETLOW, R.B. and J.K. SETLOW. 1970. Macromolecular synthesis in irradiated bacteria. Mutat. Res. 9: 434-436.

SHALEK, R.J. and T.L. GILLESPIE. 1959. Influence of oxygen upon the radiation damage of lysozyme, p.41-50. *Radiation Biology and Cancer*, University of Texas Press, Austin.

SHARMA, G.J. and P.C. KESAVAN. 1975. Use of hydrogen sulphide and *N*-ethylmaleimide in the post-irradiation modification of oxic and anoxic components of damage in *Hordeum vulgare*. Radiat. Bot. 15: 261-266.

SHENOY, M.A., J.C. ASQUITH, G.E. ADAMS, B.D. MICHAEL and M.E. WATTS. 1975. Time-resolved oxygen effects in irradiated bacteria and mammalian cells: a rapid-mix study. Radiat. Res. 62: 498-512.

SHIBATA, T., C. DasGUPTA, R.P. CUNNINGHAM and C. M. RADDING. 1979. Purified *Escherichia coli recA* protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. Proc. Natl. Acad. Sci. USA 76: 1638-1642.

SHIBATA, T., C. DasGUPTA, R.P. CUNNINGHAM, J.G.K. WILLIAMS L. OSBER and C.M. RADDING. 1981. Homologous pairing in genetic recombination: the pairing reaction catalyzed by *Escherichia coli recA* protein. J. Biol. Chem. 256: 7565-7572.

SHIMADA, K. and Y. TAKAGI. 1967. The effect of caffeine on the repair of ultraviolet-damaged DNA in bacteria. Biochim. Biophys. Acta 145: 763-770.

SHIZUYA, H. and D. DYKHUIZEN. 1972. Conditional lethality of deletions which include *uvrB* in strains of *Escherichia coli* lacking deoxynucleic acid polymerase I. J. Bacteriol. 112: 676-681.

SHLAES, D.M., J.A. ANDERSON and S.D. BARBOUR. 1972. Excision repair properties of isogenic *rec*$^-$ mutants of *Escherichia coli* K12. J. Bacteriol. 111: 723-730.

SIBATANI, A. and N. MIZUNO. 1963. Synthesis of ribonucleic acids in *Escherichia coli* irradiated with ultraviolet light. Biochim. Biophys. Acta 76: 188-200.

SLADE, H.J.K. 1983. Repair systems in *Bacteroides fragilis* Bf-2. Ph.D. Thesis, University of Cape Town, Rondebosch, Republic of South Africa.

SLADE, H.J.K., D.T. JONES and D.R. WOODS. 1981. Effect of oxygen radicals and peroxide on survival after ultraviolet irradiation and liquid holding recovery of *Bacteroides fragilis*. J. Bacteriol. 147: 685-687.

SLADE, H.J.K., J.P. SCHUMANN, J.R. PARKER, D.T. JONES and D.R. WOODS. 1983a. Effect of oxygen on host cell reactivation in *Bacteroides fragilis*. J. Bacteriol. 153: 1545-1547.

SLADE, H.J.K., J.P. SCHUMANN, D.T. JONES and D.R, WOODS. 1983b. Peroxide inducible phage reactivation in *Bacteroides fragilis*. FEMS (In press).

SLOAN, J.B. and J.E. URBAN. 1976. Growth response of *Escherichia coli* to nutritional shiftup: immediate division stimulation in slow growing cells. J. Bacteriol. 128: 302-308.

SMITH, K.C. 1969. DNA synthesis in sensitive and resistant mutants of *Escherichia coli* B after ultraviolet irradiation. Mutat. Res. 8: 481-495.

SMITH, K.C. 1978a. Multiple pathways of DNA repair in bacteria and their roles in mutagenesis. Photochem. Photobiol. 28: 121-129.

SMITH, K.C. 1978b. Molecular aspects of the interaction of far-ultraviolet radiation with living matter. *In* R.M. Tyrrell (ed.), *International Symposium on Current Topics in Radiobiology and Photobiology*. Academia Brasileira de Ciencias, Rio de Janeiro.

SMITH, K.C., and M. E. O'LEARY. 1968. The pitfalls of measuring DNA synthesis kinetics as exemplified in ultraviolet radiation studies. Biochim. Biophys. Acta 169: 430-438.

SMITH, K.C. and D.H.C. MEUN. 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on postreplication repair of damage due to ultraviolet radiation. J. Mol. Biol. 51: 459-472.

SMITH, C.L. and M. OISHI. 1978. Early events and mechanisms in the induction of bacterial SOS functions: analysis of the phage repressor inactivation process *in vivo*. Proc. Natl. Acad. Sci. USA 75: 1657-1661.

SMITH, R.D. and A.A. SALYERS. 1981. Incorporation of leucine into phospholipids of *Bacteroides thetaiotaomicron*. J. Bacteriol. 145: 8-13.

SMITH, C.J., R.A. WELCH and F.L. MACRINA. 1982. Two independent conjugal transfer systems operating in *Bacteroides fragilis* V 479-1. J. Bacteriol. 151: 281-287.

SOCRANSKY, S.S., W.J. LOESCHE, C. HUBERSAK and J.B. MacDONALD. 1964. Dependency of *Treponema microdentium* on other oral organisms for isobutyrate, polyamines, and a controlled oxidation-reduction potential. J. Bacteriol. 88: 200-209.

SOKAWA, J. and Y. SOKAWA. 1978. Relaxation effect of chloramphenicol on the stringent control in *Escherichia coli*. J. Biochem. 83: 1699-1705.

SPERRY, J.F., M.D. APPLEMAN and T.D. WILKINS. 1977. Requirements of heme for growth of *Bacteroides fragilis*. Appl. Environ. Microbiol. 34: 386-390.

SRIVASTAVA, B.S. 1976. Irradiation and DNA breaks. Nature 259: 425-426.

STADTMAN, T.C. 1980. Selenium-dependent enzymes. Annu. Rev. Biochem. 49: 93-110.

STALLIONS, D.R. and R. CURTISS. 1972. Bacterial conjugation under anaerobic conditions. J. Bacteriol. 111: 294-295.

STEINBORN. G. 1979. *uvm* Mutant of *Escherichia coli* K12 and UV mutagenesis. Mol. Gen. Genet. 175: 203-208.

STEINMAN, H.M. 1978. The amino acid sequence of mangano superoxide dismutase from *Escherichia coli* B. J. Biol. Chem. 253: 8708-8720.

STEPHENS, J.C., S.W. ARTZ and B.N. AMES. 1975. Guanosine 5' diphosphate 3' diphosphate (ppGpp): positive effector for histidine operon transcription and general signal for amino acid deficiency. Proc. Natl. Acad. Sci. USA 72: 4389-4393.

STEVENSON, R.M.W. 1979. Amino acid uptake systems in *Bacteroides ruminicola*. Can. J. Microbiol. 25: 1161-1168.

STIFFLER, P.W., R. KELLER and N. TRAUB. 1974. Isolation and characterization of several cryptic plasmids from clinical isolates of *Bacteroides fragilis*. J. Infect. Dis. 130: 544-548.

STRIKE, P. and P.T. EMMERSON. 1974. Degradation of DNA following ultraviolet irradiation of *Escherichia coli* K12 mutants lacking DNA polymerase I and exonuclease V. Mol. Gen. Genet. 130: 39-45.

SUSSMAN, R., J. RESNICK, K. CALAME and J. BALUCH. 1978. Interaction of bacteriophage λ repressor with nonoperator DNA containing single-strand gaps. Proc. Natl. Acad. Sci. USA 75: 5817-5821.

SUZUKI, K. and Y. IWAMA. 1960. Recovery in the presence of chloramphenicol of *Escherichia coli* B (H) irradiated with ultraviolet light. Nature 187: 347-348.

SWARTZ, H.M. 1971. Effect of oxygen on freezing damage. II. Physical-chemical effects. Cryobiology 8: 255-264.

SWEET, D.M. and B.E.B. MOSELEY. 1974. Accurate repair of ultraviolet-induced damage in *Micrococcus radiodurans*. Mutat. Res. 23: 311-318.

SWEET, D.M. and B.E.B. MOSELY. 1976. The resistance of *Micrococcus radiodurans* to killing and mutation by agents which damage DNA. Mutat. Res. 34: 175-186.

SWENSON, P.A. 1972. Release of the β-galactosidase-synthesizing system from ultraviolet catabolite repression by cyclic 3',5'-adenosine monophosphate, dark repair, photoreactivation, and cold treatment. J. Bacteriol. 109: 391-398.

SWENSON, P.A. 1976. Physiological responses of *Escherichia coli* to far-ultraviolet radiation, p.269-387. *In* K.C. Smith (ed.), *Photochemical and photobiological reviews*, Vol.1. Plenum Press, New York.

SWENSON, P.A. 1978. Death of *Escherichia coli* cells as a consequence of damage to DNA by far-ultraviolet radiation, p.783-788. *In* P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), *DNA repair mechanisms*. Academic Press, New York.

SWENSON, P.A. and R.B. SETLOW. 1964. β-Galactosidase: inactivation of its messenger RNA by ultraviolet irradiation. Science 146: 791-794.

SWENSON, P.A. and R.B. SETLOW. 1966. Effects of ultraviolet radiation on macromolecular synthesis in *Escherichia coli*. J. Mol. Biol. 15: 201-219.

SWENSON, P.A. and R.L. SCHENLEY. 1970a. Evidence for the control of respiration by DNA in ultraviolet-irradiated *Escherichia coli* B/r cells. Mutat. Res. 9: 443-453.

SWENSON, P.A. and R.L. SCHENLEY. 1970b. Role of pyrimidine nucleotides in the control of respiration in ultraviolet-irradiated *Escherichia coli* B/r cells. J. Bacteriol. 104: 1230-1235.

SWENSON, P.A. and R.L. SCHENLEY. 1972. Death through respiratory failure of a fraction of ultraviolet-irradiated *Escherichia coli* B/r cells. J. Bacteriol. 111: 658-663.

SWENSON, P.A. and R.L. SCHENLEY. 1974a. Respiration, growth and viability of repair-deficient mutants of *Escherichia coli* after ultraviolet irradiation. Int. J. Radiat. Biol. 25: 51-60.

SWENSON, P.A. and R.L. SCHENLEY. 1974b. Evidence relating cessation of respiration, cell envelope changes, and death in ultraviolet-irradiated *Escherichia coli* B/r cells. J. Bacteriol. 117: 551-559.

SWENSON, P.A., R.L. SCHENLEY and J.G. JOSHI. 1977. Role of cyclic adenosine 3'-5'-monophosphate on cessation of respiration in ultraviolet-irradiated *Escherichia coli*. J. Bacteriol. 131: 707-709.

SWENSON, P.A., J.G. JOSHI and R.L. SCHENLEY. 1978. Regulation of septation, respiration and killing by cyclic 3'-5'-adenosine-monophosphate and its receptor protein after far-ultraviolet irradiation of *Escherichia coli*. Mol. Gen. Genet. 159: 125-130.

TAIT, R.C., A.L. HARRIS and D.W. SMITH. 1974. DNA repair in *Escherichia coli* mutants deficient in DNA polymerases I, II and/or III. Proc. Natl. Acad. Sci. USA 71: 675-679.

TALLENTIRE, A. 1981. Oxygen-dependent damaging processes in irradiated cells, p.247-262. *In* M.A.J. Rogers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

TALLENTIRE, A. and E.L. POWERS. 1963. Modification of sensitivity to X-irradiation by water in *Bacillus megaterium*. Radiat. Res. 20: 270-287.

TALLY, F.P., P.R. STEWART, V.L. SUTTER and J.E. ROSENBLATT. 1975. Oxygen tolerance of fresh clinical anaerobic bacterial J. Clin. Microbiol. 1: 161-164.

TALLY, F.P. , B.R. GOLDIN, N.B. JACOBUS and S.L. GORBACH. 1977. Superoxide dismutase in anaerobic bacteria of clinical significance. Infect. Immun. 16: 20-25.

TALLY, F.P., D.R. SNYDMAN, S.L. GORBACH and M.H. MALAMY. 1979. Plasmid mediated transferable resistance to clindamycin and erythromycin in *Bacteroides fragilis*. J. Infect. Dis. 139: 83-88.

TALLY, F.P., M.J. SHEMELL, G.R. CARSON and M.H. MALAMY. 1981. Chromosomal and plasmid-mediated transfer of clindamycin resistance in *Bacteroides fragilis*, p. 51-59. *In* S.B. Levy, R.C. Clowes and E.L. Koenig (eds.), *Molecular biology, pathogenicity, and ecology of bacterial plasmids*. Plenum Publishing Corp., New York.

TANG, M. and M.H. PATRICK. 1977a. Repair of UV damage in *E.coli* under non-growth conditions. Photochem. Photobiol. 26: 247-255.

TANG, M. and M.H. PATRICK. 1977b. The role of DNA polymerase I in liquid holding recovery of UV-irradiated *E.coli*. Photochem. Photobiol. 26: 257-262.

TAYLOR, J.E., J.F. YAN and J. WANG. 1966. The iron (III)-catalyzed oxidation of cysteine by molecular oxygen in the aqueous phase. An example of a two-thirds order reaction. J. Am. Chem. Soc. 88: 1663-1667.

TAYLOR, W. and W. GINOZA. 1967. Correlation of γ-ray inactivation and strand scission in the replicative form of ØX174 bacteriophage DNA. Proc. Natl. Acad. Sci. USA 58: 1753-1757.

TAYLOR, A. and G.R. SMITH. 1980. Unwinding and rewinding of DNA by the *recBC* enzyme. Cell 22: 447-457.

TETU, C., E. DASSA and P. BOQUET. 1980. The energy dependent degradation of guanosine 5'-diphosphate 3'-diphosphate in *Escherichia coli*. Lack of correlation with ATP levels *in vivo* and role of the transmembrane proton gradient. Eur. J. Biochem. 103: 117-124.

THODAY, J.M. and J. READ. 1947. Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. Nature 160: 608-609.

THOMPSON, J.K. and M.G.R. HART. 1981. Novel patterns of ultraviolet mutagenesis and Weigle reactivation in *Staphylococcus aureus* and phage Ø11. J. Gen. Microbiol. 124: 147-157.

TINNELL, W.H. and F.L. MACRINA. 1976. Extrachromosomal elements in a variety of strains representing the *Bacteroides fragilis* group of organisms. Infect. Immun. 14: 955-964.

TOMILIN, N.V. and S.V. ZHEREBTSOV. 1982. An analysis of the repair processes in ultraviolet-irradiated *Micrococcus luteus* using purified ultraviolet-endonuclease. Biochem. Biophys. Acta 698: 287-294.

TOTTER, J.R. 1981. Some reflections on oxygen and oxy-radicals in chemistry and biology, p.1-13. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

TOWN, C.D., K.C. SMITH and H.S. KAPLAN. 1971. DNA polymerase required for rapid repair of X-ray-induced DNA strand breaks *in vivo*. Science 172: 851-854.

TOWN, C.D., K.C. SMITH and H.S. KAPLAN. 1972. Influence of ultrafast repair processes (independent of DNA polymerase I) on the yield of DNA single-strand breaks in *E.coli* K12 X-irradiated in the presence or absence of oxygen. Radiat. Res. 52: 99-114.

TOWN, C.D., K.C. SMITH and H.S. KAPLAN. 1973. The repair of DNA single-strand breaks in *E.coli* K12 X-irradiated in the presence or absence of oxygen; the influence of repair on cell survival. Radiat. Res. 55: 334-345.

TRGOVČEVIĆ, Ž., D. PETRANOVIĆ, M. PETRANOVIĆ and E. SALAJ-ŠMIC. 1980. *recA* gene product is responsible for inhibition of deoxyribonucleic acid synthesis after ultraviolet irradiation. J. Bacteriol. 143: 1506-1508.

TRGOVCEVIĆ, Ž., E. SALAJ-ŠMIC, M. PETRANOVIĆ and D. PETRANOVIĆ. 1981. Programmed death of *Escherichia coli* after UV radiation, p.505-510. *In* E. Seeberg and K. Kleppe (eds.), *Chromosome damage and repair.* Plenum Publishing Corp., New York.

TYRRELL. R.M. 1973. Suppression of photoreactivating enzyme production in *Escherichia coli* grown under anaerobic conditions. J. Bacteriol. 115: 450-452.

UCHIDA, Y., H. SHIGEMATSU and K. YAMAFUGI. 1965. The mode of action of hydrogen peroxide on deoxyribonucleic acid. Enzymologia 29: 369-376.

UHLIN, B.E. and A.J. CLARK. 1981. Overproduction of the *Escherichia coli recA* protein without stimulation of its proteolytic activity. J. Bacteriol. 148: 386-390.

UPTON, A.C. 1982. The biological effects of low-level ionizing radiation. Sci. Am. 246: 29-37.

VANCE, P.G., B.B. KEELE, Jr. and K.V. RAJAGOPALAN. 1972. Superoxide dismutase from *Streptococcus mutans*. Isolation and characterization of two forms of the enzyme. J. Biol. Chem. 247: 4782-4786.

VAN HEMMEN, J.J., W.J.A. MEULING, J. DE JONG and L.H. LUTHJENS. 1974. Radioprotection of biologically-active DNA by cysteamine: a rapid-mix study. Int. J. Radiat. Biol. 25: 455-464.

VAN HEMMEN, J.J., W.J.A. MEULING and J.F. BLEICHRODT. 1978. Effect of oxygen on inactivation of biochemically active DNA by γ rays *in vitro*. Influence of metalloporphyrins and enzymatic DNA repair. Radiat. Res. 75: 410-423.

VAN TASSELL, R.L. and T.D. WILKINS. 1978. Isolation of auxotrophs of *Bacteroides fragilis*. Can. J. Microbiol. 24: 1619-1621.

VAREL, V.H. and M.P. BRYANT. 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl. Microbiol. 18: 251-257.

VILLANI, G., S. BOITEUX and M. RADMAN. 1978. Mechanism of ultraviolet-induced mutagenesis: extent and fidelity of *in vitro* DNA synthesis on irradiated templates. Proc. Natl. Acad. Sci. USA 75: 3037-3041.

VILLAREJO, M. and J. WESTLEY. 1966. Sulfur metabolism of *Bacillus subtilis*. Biochim. Biophys. Acta 117: 209-216.

VOLKERT, M.R., D.F. SPENCER and A.J. CLARK. 1979. Indirect and intragenic suppression of the *lexA102* mutation in *Escherichia coli* B/r. Mol. Gen. Genet. 177: 129-137.

VOLKERT, M.R., L.J. MARGOSSIAN and A.J. CLARK. 1981. Evidence that *rnmB* is the operator of the *Escherichia coli recA* gene product. Proc. Natl. Acad. Sci. USA 78: 1786-1790.

WAINFAN, E., L.R. MANDEL and E. BOREK. 1963. Differential inhibition of the synthesis of soluble and particulate RNA. Biochem. Biophys. Res. Commun. 10: 315-322.

WALDEN. W.C. and D.J. HENTGES. 1975. Differential effects of oxygen and oxidation-reduction potentials on the multiplication of three species of anaerobic intestinal bacteria. Appl. Microbiol. 30: 781-785.

WALKER. G.C. 1978. Mutagenesis- and repair-enhancing activities associated with the plasmid pKM101. Cold Spring Harbor Symp. Quant. Biol. 43: 893-896.

WALKER, G.C. and P.P. DOBSON. 1979. Mutagenesis and repair deficiencies of *Escherichia coli umuC* mutants are suppressed by the plasmid pKM101. Mol. Gen. Genet. 172: 17-24.

WARD, D.F. and N.E. MURRAY. 1980. Construction and characterization of *Escherichia coli polA-lacZ* gene fusions. J. Bacteriol. 142: 962-972.

WATSON, J.D. 1952. The properties of X-ray inactivated bacteriophage. J. Bacteriol. 63: 473-485.

WEBB, R.B. 1970. Two classes of postirradiation oxygen effect in *Staphylococcus aureus*, p.93-94. Annual report, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois.

WEBB, R.B. 1977. Lethal and mutagenic effects of near-ultraviolet radiation, p.169-261. *In* K.C. Smith (ed.), *Photochemical and photobiological reviews*, Vol.2. Plenum Publishing Corp., New York.

WEBB, R.B. and J.R. LORENZ. 1970. Oxygen dependence and repair of lethal effects of near UV and visible light. Photochem. Photobiol. 12: 283-289.

WEINSTOCK, G.M., K. McENTEE and I.R. LEHMAN. 1979. ATP-dependent renaturation of DNA catalyzed by the *recA* protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 76: 126-130.

WEISIGER, R.A. and I. FRIDOVICH. 1973. Superoxide dismutase. Organelle specificity. J. Biol. Chem. 248: 3582-3592.

WEISS, H. and A. SANTOMASSO. 1981. The effect of oxygen, chemical agents, dose rate and temperature on the radiation response of wet spores, p.263-268. *In* M.A.J. Rodgers & E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*, Academic Press, New York.

WEITZMAN, S.A. and T.P. STOSSEL. 1981. Mutation caused by human phagocytes. Science 212: 546-547.

WELCH, R., K.R. JONES and F.L. MACRINA. 1979. Transferable lincosamide-macrolide resistance in *Bacteroides*. Plasmid 2: 261-268.

WEST, S.C. and P.T. EMMERSON. 1977. Induction of protein synthesis in *E.coli* following UV or γ irradiation, mitomycin C treatment or *tif* expression. Mol. Gen. Genet. 151: 57-67.

WILKINS, T.D., D.L. WAGNER, B.J. VELTRI, Jr. and E.M. GREGORY. 1978. Factors affecting production of catalase by *Bacteroides*. J. Clin. Microbiol. 8: 553-557.

WILLIAMS, J.G.K., T. SHIBATA and C.M. RADDING. 1981. *Escherichia coli recA* protein protects single-stranded DNA or gapped duplex DNA from degradation by *recBC* DNase. J. Biol. Chem. 256: 7573-7582.

WILLETTS, N.S. and A.J. CLARK. 1969. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. J. Bacteriol. 100: 231-239.

WILLIS, A.T. 1969. Techniques for the study of anaerobic spore forming bacteria, p.79-115. *In* J.R. Norris and D.W. Ribbons (eds.), *Methods in Microbiology*, Vol.3B. Academic Press, London.

WILLSON, R. 1981. Free radicals, oxygen and radio-sensitising drugs: a very brief introduction, p.309-320. *In* M.A.J. Rodgers and E.L. Powers (eds.), *Oxygen and oxy-radicals in Chemistry and Biology*. Academic Press, New York.

WILSON, C.B., V. TSAI and J.S. REMINGTON. 1980. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. J. Exp. Med. 151: 328-346.

WIMPENNY, J.W.T. and O.A. SAMAH. 1978. Some effects of oxygen on the growth and physiology of *Selenomonas ruminantium*. J. Gen. Microbiol. 108: 329-332.

WITKIN, E.M. 1959. Post-irradiation metabolism and the timing of ultraviolet-induced mutations in bacteria, p.280-299. *In* Proceedings of the X International Congress on Genetics, Vol.I.

WITKIN, E.M. 1967. The radiation sensitivity of *Escherichia coli* B: a hypothesis relating filament formation and prophage induction. Proc. Natl. Acad. Sci. USA 57: 1275-1279.

WITKIN, E.M. 1975. Persistence and decay of thermo-inducible error-prone repair activity in nonfilamentous derivatives of *tif-1 Escherichia coli* B/r: the timing of some critical events in ultraviolet mutagenesis. Mol. Gen. Genet. 142: 87-103.

WITKIN, E.M. 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. 40: 869-907.

WITTE, W. and H. BÖHME. 1972. The action of caffeine on the survival of *Proteus mirabilis* and its virulent phage VI_r after UV-irradiation and treatment with nitrogen mustard. Mutat. Res. 16: 133-139.

YAMAFUGI, K. and Y. UCHIDA. 1966. Liberation of adenine from deoxyribonucleic acid by hydrogen peroxide. Nature 209: 301-302.

YOAKUM, G. and A. EISENSTARK. 1972. Toxicity of L-tryptophan photoproduct on recombinationless mutants of *Salmonella typhimurium*. J. Bacteriol. 112: 653-655.

YOAKUM, G.H. and L. GROSSMAN. 1981. Identification of *E.coli uvrC* protein. Nature 292: 171-173.

YONEI, S. and K. NOZU. 1972. Mechanism of post-irradiation degradation of deoxyribonucleic acid in a radiosensitive *Escherichia coli* (NG 30) irradiated with ultraviolet light. J. Mol. Biol. 65: 213-225.

YOST, F.J., Jr. and I. FRIDOVICH. 1973. An iron-containing superoxide dismutase from *Escherichia coli*. J. Biol. Chem. 248: 4905-4908.

YOST, F.J. and I. FRIDOVICH. 1976. Superoxide and hydrogen peroxide in oxygen damage. Arch. Biochem. Biophys. 175: 514-519.

YOUNGS, D.A. and I.A. BERNSTEIN. 1973. Involvement of *recB-recC* nuclease (exonuclease V) in the process of X-ray-induced deoxyribonucleic acid degradation in radiosensitive strains of *Escherichia coli* K-12. J. Bacteriol. 113: 901-906.

YOUNGS, D.A. and K.C. SMITH. 1973. Evidence for the control by *exrA* and *polA* genes of two branches of the *uvr* gene-dependent excision repair pathway in *Escherichia coli* K-12. J. Bacteriol. 116: 175-182.

YOUNGS, D.A., E. VAN DER SCHEUREN and K.C. SMITH. 1974. Separate branches of the *uvr* gene-dependent excision repair process in ultraviolet-irradiated *Escherichia coli* K-12 cells; their dependence upon growth medium and the *polA*, *recA*, *recB* and *exrA* genes. J. Bacteriol. 117: 717-725.

ZETTERBERG, G. 1964. Mutagenic effects of UV and visible light, p.247-281. *In* A.C. Giese (ed.), *Photophysiology*, Vol.3. Academic Press, Inc., New York.

ZHEREBTSOV, S.V. and N.V. TOMILIN. 1982. The roles of different repair mechanisms in the ultraviolet resistance of *Micrococcus luteus*. Biochim. Biophys. Acta 698: 295-302.